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ELEMENT-BUILDING REACTIONS IN STARS

By William A. Fowler

KELLOGG RADIATION LABORATORY, CALIFORNIA INSTITUTE OF TECHNOLOGY

AND Jesse L. Greenstein

MOUNT WILSON AND PALOMAR OBSERVATORIES
CARNEGIE INSTITUTION OF WASHINGTON
CALIFORNIA INSTITUTE OF TECHNOLOGY

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Considerable evidence exists for the "beginning" of the universe and the atoms, as we now know them, five billion years ago. The age of the atoms of naturally radioactive elements, the age of the oldest known stars, and the time scale for the expansion of the universe approximately coincide. This singular event in time has considerably affected theories of the origin of the chemical elements; at an early phase in the expansion, the density and temperature might have been very high, and some dynamic equilibrium may have existed between radiation, nucleons, and heavy nuclei. As the expansion proceeded, after a short time a considerable fraction of the mass of the universe would have existed as neutrons, which built the heavy nuclei by a succession of capture and beta-decay processes. The frozen-in effects of the chain of neutron captures have been studied by Gamow, Alpher, and Herman; they propose a detailed model of the expansion and element-building and predict moderately well some features of the observed relative abundances of the elements in the earth and normal stars. Excellent summaries of some older hypotheses on the origin of the elements are found in the *Reviews of Modern Physics*, Vol. **22**, 1950.

In this article we address ourselves in a nontechnical fashion to the following limited questions: Can nuclear and astrophysical processes, now understood, be found which produce the heavier elements from hydrogen, in stars, under conditions such as exist at present? Is there any astrophysical evidence that element-building now occurs? We are not committed to any speculative relativistic cosmology, nor are we excluding the possibility of a primeval, explosive phase. We wish to trace the evolution of a universe containing initially only the simplest atom, hydrogen, part of which condenses into stars, leaving an interstellar gas, out of which later generations of stars may condense. We will show that known laws of nuclear physics and known astronomical processes lead to element-building in certain stars; mass loss to interstellar space, or explosion, both of which now occur, returns heavier elements to be mixed with the interstellar hydrogen. New stars

formed will have an increased proportion of heavy elements. Observational checks may exist, therefore, in the change of the ratio of hydrogen to heavier elements, dependent on the age of the stars. If, in certain cases, matter can be circulated from the hot core to the surface of a star, the consequences of nuclear synthesis may become observable in stellar spectra which show anomalous abundances of the elements. It should be noted that astrophysical investigation shows that the compositions of the sun and of recently formed stars are more or less similar, that hydrogen and helium form almost 98 per cent of the mass of stellar material, that the ratio hydrogen/metals is about 10,000 by number of atoms, and that a general "abundance curve" seems to exist, connected both with the formation processes and the stability properties of the nuclei. We will be concerned mainly with the synthesis of the 2 per cent by mass of the elements heavier than He, which we will loosely call "metals."

If a high-density phase of the universe synthesized heavier elements from neutrons and protons in the "beginning," then, on our point of view, the resulting abundance distribution will have been considerably modified by nuclear reactions in stellar interiors over the subsequent history of the universe. We will not discuss the high-density phase at all but will limit ourselves to the possibilities of building the heavier elements from elemental hydrogen without recourse to such a high-density phase.

Our primary motivation in this problem has been twofold. First, we have wished to enumerate those nuclear reactions which probably occur in stars in the processes of energy generation and element synthesis. These ought to be subjected to rigorous experimental testing in the nuclear physics laboratory, to establish or confirm their existence and to determine their detailed quantitative properties. In particular, their cross-sections as a function of energy permit reaction rates as a function of temperature and density to be accurately calculated. Second, we have sought to ascertain what astronomical observations now give evidence for continuing nuclear processes in stars and what further experimental and theoretical studies should be made in what might be termed "astronuclear physics." Stellar interiors provide a locale for fusion processes which difficulty of containment and too short duration of laboratory experiments otherwise render unobservable.

The process of gravitational contraction of a protostar containing only H will lead to a temperature rise in the interior, and when $T \sim 5$ million degrees, the proton-proton chain of thermonuclear fusion processes occurs, synthesizing D^2, He^3, He^4, as follows:

$$H^1(p, \beta^+\nu)D^2(p, \gamma)He^3(He^3, 2p)He^4. \qquad (1)$$

In the last stage the He^3, which does not interact with H^1 or D^2, builds up in concentration until enough encounters between pairs of He^3 nuclei occur to complete the synthesis of He^4.

The first of the reactions listed above is the primary mechanism by which element synthesis is initiated, using hydrogen atoms as universal building blocks, as postulated in this article. It is one of the few nuclear reactions discussed here which has not been experimentally observed. It is a "prompt" beta-decay reaction which is allowed only by the Gamow-Teller mechanism of electron-neutrino processes. It is now known that many allowed beta processes proceed through

this mechanism, and there is thus excellent theoretical justification for the existence of direct proton-proton synthesis of deuterium and for the rate of the process as calculated using modern beta-decay theory. Now that experiments are under way at Los Alamos to detect the absorption of neutrinos in light hydrogen, it may soon be possible to search for their absorption in deuterium. This might lead to the detection of the first of reactions (1) in the reverse direction, i.e., deuterium → two protons plus an electron, but this would require that neutrinos and antineutrinos be identical. If this is not the case, the "mirror" process—deuterium → two neutrons plus a positron—will be observed. Observation of the mirror process would constitute fundamental experimental confirmation of the very general ideas underlying the proton-proton process.

Energy production occurs in the core of the star; from astrophysical observations it appears that the reaction products are mixed into the outer envelope only with extreme difficulty, and probably not until the H is substantially exhausted in the core. As the core increases in size, if the star has a mass several times that of the sun, the interior cannot support the weight of the envelope, and, rather than cooling, the star contracts and the temperature increases further. At about 150 million degrees, the fusion of helium begins:

$$He^4 + He^4 \rightleftharpoons Be^8. \tag{2}$$

Be^8 does not exist as an isotope of beryllium in nature, and when produced in the laboratory, even in its lowest quantum state, it disintegrates into two alpha particles, with an energy release of approximately 90 kev. Thus reaction (2) does not go to completion but at a given temperature and density results in an equilibrium abundance of Be^8 given essentially by a Maxwell-Boltzmann factor. For helium at 150 million degrees and a density of 10^4 gm./cc. the ratio of Be^8 nuclei to He^4 nuclei is approximately 10^{-9}. Stable nuclei capture alpha particles with the emission of gamma radiation. Be^8, with its relatively long lifetime so far as nuclear processes are concerned (theoretically, $\sim 10^{-16}$ seconds; experimentally, 10^{-16}–10^{-14} seconds), ought to be no exception. The process

$$Be^8 + He^4 \rightarrow (C^{12}) \rightarrow C^{12} + \gamma \tag{3}$$

cannot be initiated in the laboratory for lack of a Be^8 target, but it has recently been shown in the Kellogg Radiation Laboratory that an excited state of C^{12}, available as the resonant compound nucleus (C^{12}) in the thermal interaction of Be^8 and He^4 in stars, can be produced in the laboratory and that both alpha decay and gamma decay are observed for this state. The reversibility of nuclear reactions then indicates that reaction (3) is a valid nuclear process, resonant at the central temperature of giant stars.

The formation of C^{12} is followed by successive alpha-particle captures, so that one has the following chain of (α, γ) reactions:

$$Be^8(\alpha, \gamma)C^{12}(\alpha, \gamma)O^{16}(\alpha, \gamma)Ne^{20} \ldots . \tag{3'}$$

The chain probably terminates at Ne^{20} because of the exhaustion of the helium. The result is a mixture of C^{12}, O^{16}, and Ne^{20} in relative abundances determined by the rates of the reactions given in expression (3′).

The heavier elements in this chain can be synthesized only at increasingly higher temperatures. Note that the nonexistence of stable nuclei of masses 5 and 8 formed an insuperable obstacle to the older neutron-capture theories; the instability of mass 8 gives no difficulty here, since, with stellar densities and lifetimes, even the low concentration given by reaction (2) suffices for ample C^{12} formation so long as resonance occurs in reaction (3).

The exhaustion of the helium by reactions (3) will be followed by further condensation and temperature rise for the core of those stars which remain stable. At sufficiently high temperature, reactions among the C, O, and Ne nuclei will take place. As a final stage, equilibrium will be established in all possible nuclear processes at about 5 billion degrees, and the result will be the formation of the most stable nuclei near the minimum of the packing-fraction curve, namely, iron and the nearby elements. With the production of these nuclei, no further release of energy is possible from nuclear processes. It is suggested that the marked peak in the universal abundance curve, symmetric about Fe^{56}, is due to those stars which remained stable until all nuclear energy release had terminated.

Now let us return to a star which has synthesized C^{12}, O^{16}, and Ne^{20} but becomes unstable at this point, explodes, and mixes its matter with the interstellar gas. In time, new stars formed will contain these elements as well as fresh hydrogen. This leads to a synthesis of great importance, through the Bethe carbon-nitrogen cycle (operating at about 15 million degrees in the core) and eventually through higher temperature processes involving O and Ne. The reactions involved are:

$$C^{12}(p, \gamma)N^{13}(\beta^+\nu)C^{13}(p, \gamma)N^{14}(p, \gamma)O^{15}(\beta^+\nu)N^{15}(p, \alpha)C^{12}, \tag{4}$$

with some small leakage through $N^{15}(p, \gamma)O^{16}$ to the oxygen isotopes:

$$O^{16}(p, \gamma)F^{17}(\beta^+\nu)O^{17}(p, \alpha)N^{14}. \tag{5}$$

(Note that the oxygen nuclei return to N^{14} and thus to the carbon-nitrogen isotopes. A generalized catalytic cycle includes CNO isotopes.) The reaction of the neon-sodium cycle are:

$$Ne^{20}(p, \gamma)Na^{21}(\beta^+\nu)Ne^{21}(p, \gamma)Na^{22}(\beta^+\nu)Ne^{22}(p, \gamma)Na^{23}(p, \alpha)Ne^{20}. \tag{6}$$

The group of reactions (4), (5), and (6) constitute the source of energy in the hotter, second-generation stars, catalytically converting hydrogen into helium. At the same time they constitute a mechanism for synthesizing the other stable isotopes of C, N, O, and Ne, as well as Na^{23}. Among others, the very important nuclei, C^{13}, O^{17}, and Ne^{21} are formed and exist in appreciable equilibrium concentrations (e.g., $C^{13}/C^{12} = 1/4$). These stable isotopes have in common one excess neutron, as compared with the most stable light elements. If stellar material containing C^{13}, O^{17}, and Ne^{21} exhausts its hydrogen by normal thermonuclear processes, helium burning begins again, but at a lower temperature, 85 million degrees. This is reached in the giant stage of "second-generation" stars, and instead of reactions (2) and (3) the following occur:

$$\begin{aligned} &C^{13}(\alpha, n)O^{16}, \\ &O^{17}(\alpha, n)Ne^{20}, \\ &Ne^{21}(\alpha, n)Mg^{24}. \end{aligned} \tag{7}$$

Reactions (7) are important less as energy sources than as sources of free neutrons. These neutrons can be captured and in successive stages build the metals and heavy elements. The temperatures, corresponding to $kT \sim 10$ kev., and the densities suffice for rapid heavy-element formation up to Pb.

The neutrons will be thermalized and captured by other nuclei before they decay to protons at the densities of stellar interiors. Each radiative-capture process increases the atomic weight of the capturing nucleus by unity. In a star containing the normal abundance peak of the elements near Fe^{56}, most of the captures will be made by these elements and their successively heavier products In this way all the heavy elements up to the naturally radioactive alpha-emitters can be synthesized. Some of the latter emit alpha particles so rapidly that no synthesis beyond Pb^{208} and Bi^{209} is possible. In stars which contained only the lightest element, say, H, He, C, N, O, and Ne, it will be these elements on which the synthesis is based; it is, in fact, probable that the elements from Na to Ti were synthesized in this manner.

The temperatures in the cores of giant stars correspond to $kT \sim 10$ kev., and it is neutron-capture cross-sections near this energy which are relevant in the buildup process. The relative abundances of the elements produced will depend on the neutron flux, the time scale, and these cross-sections. Preliminary calculations based on the scanty experimental values available indicate some agreement with the relative cosmical abundances.

The abundance distribution of the elements and their isotopes suggests that neutron capture processes have occurred both on fairly long time scales as in the evolution of giant stars and on relatively short time scales as in supernova explosions. On a very short time scale it is even possible that the parents of the natural alpha-emitters have been synthesized by neutron capture.

The processes discussed so far occurring in stellar interiors will synthesize, from hydrogen and its first product, helium, all the known nuclei from C to Bi. However, as noted above, the elements from Bi^{209} up to the parents of the naturally radioactive alpha-emitters cannot be produced in this way except on a very short time scale. Furthermore, deuterium and the isotopes of Li, Be, and B are not synthesized but are destroyed at the central temperatures of even the cooler main-sequence stars. For this reason, it has been suggested that these elements may be produced on the surfaces of stars in magnetic areas similar to sunspots. In areas of changing magnetic fields betatron action accelerates particles to much higher energies than are possible in the quiescent surface or even in the interior. The emission of low-energy cosmic radiation from stars may also be attributed to this acceleration mechanism. A reasonable case can be made for the production of D, Li, Be, and B and even of the alpha-emitters under the extreme conditions to be expected in these active spots.

If the enriched heavier material produced in rapidly evolving stars is spread through interstellar space, subsequently formed stars will resemble in composition the oldest known stars, and if the process continues for any length of time, the newer stars will show a larger metal/hydrogen ratio.

Thus in a few generations, during the early phases of star formation, matter with an increasingly high abundance of the heavy elements will be found in space. The conditions in stellar interiors can be calculated; the reaction rates can be de-

termined from laboratory experiments and theory. In short, the elements may be formed under fairly well-known conditions. Where the reaction rates are known, relative concentrations of the elements and isotopes can be predicted as a function of temperature. In addition, unstable isotopes are now being formed in some stars, and atoms younger than those now existing on the earth might be observable. We may now ask whether any effects of reactions (1) through (7) are observable and whether there is any astrophysical evidence for the evolution of the elements.

One important feature of the theory of stellar interiors is that high central temperatures can be attained only in massive stars, when the hydrogen energy source is exhausted, and if there is little or no mixing between the helium core and the hydrogen-rich envelope. This lack of mixing is well established for the sun, where spectroscopic investigation shows that the relative abundances of C^{12}:C^{13}:N^{14} do not conform to predictions based on the carbon cycle (4) and that easily destroyed elements like Li and Be exist at the surface. Let us imagine, however, that at certain stages in evolution, when energy sources are exhausted or when mass is lost because of internal or surface instability, mixing does occur and brings the reaction products to the surface. Then the reactions lead to predicted changes of relative abundances from those in normal stars. These may be checked by spectroscopic analysis of stellar atmospheres.

The great apparent variety of the spectra of normal stars has been shown to be representable by one major spectral sequence. Differences of temperature (the primary parameter) and of surface gravity (or pressure), will explain most stellar spectra observed, with nearly constant elemental abundances from star to star. However, certain stars do stand outside the normal spectral sequence, and most observed peculiarities can be explained as a result of nuclear reactions. Some examples follow. In both normal energy-producing reactions (1) and (4), H is converted into He. Thus stars which have substantially exhausted their H should exist, be He-rich, and show traces of the detailed predictions of the reactions. For example, at solar-core temperatures, the ratio $N^{14}/C^{12} = 20$ (and drops to about 4 at very high central temperatures). Thus exhausted stars should be He- and N-rich. Both these predictions are observationally verified in subluminous hot subdwarfs and in some supergiants. The predictions of formula (1) are unfortunately not otherwise verifiable, since D^2 and He^3 exist in negligible concentrations. But those of formula (4) seem verifiable in detail. The terrestrial concentration $C^{13}/C^{12} \backsimeq {}^1/_{90}$, but in certain red giant stars the predicted value ${}^1/_4$ is actually observed. (These stars are also carbon-rich objects; the C^{13} is probably synthesized when C^{12} produced in the core traverses a hot hydrogen shell around the core.) The existence of carbon-rich stars is one of the arguments for the occurrence of reactions involving helium burning (reactions [3]). In these stars H is very deficient, He is probably superabundant, and N, O, may also be enhanced. Be^8 is obviously not observable, and no evidence for large O, Ne, or Mg excess exists. The most direct evidence for heavy-element building is found in the red-giant group, which on current theories of stellar structure have dense, very hot cores surrounded by a large low-density envelope. Most carbon-rich stars are red giants, as are the most peculiar objects, the S stars. The latter were first recognized by bands of ZrO (as contrasted to TiO for normal cool stars). They prove also to have bands of LaO, GdO, and CbO, and stronger than normal atomic lines of the elements of

the fifth and sixth periods, as contrasted to the iron-group elements. But the most convincing argument for contemporary element synthesis is the existence, in the S-type stars, of Tc, the unstable element whose most stable known isotope has a lifetime of 200,000 years. Our interpretation is that neutron-capture processes are now synthesizing the heavy elements and Tc in the core, as a consequence of reactions (7), which produce neutrons in ample numbers; the mixing time must be less than a million years if the Tc is to survive as far as the surface.

If we believe that appreciable element synthesis is now occurring, we may extrapolate into the past and expect that stars formed before the sun would show appreciable abundance deficiencies for elements heavier than He. One of the fundamental difficulties is that we do not understand the original mechanics of star formation or the rate as a function of time. Since the composition of the sun does not differ greatly from that of recently formed stars, it is reasonable to assume that the current rate of heavy-element synthesis is low. The age of the earth and meteorites is approximately 4.6×10^9 years, and from the decay of I^{129} to Xe^{129} there is some evidence that not less than 0.4×10^9 years elapsed between the formation of the I^{129} atoms and their crystallization in meteorites. Thus some time, even if an uncomfortably short one, seems to exist before the formation of the sun. Speculatively, we may assume that a much larger number of massive stars were formed in the first half-billion years of the existence of the universe than have been formed since. These massive stars evolved rapidly. In this same period, the larger part of the oldest stars were also formed; the sun and stars like it formed near the close of this period of the formation of both stars and heavy atoms. While stars continue to be formed, the amount of interstellar gas and the rate of star and heavy-atom formation seem to be relatively lower, at present, but not negligible.

If there existed an epoch in which formation of heavy nuclei and of most of the stars was proceeding rapidly, then we might expect to find gross differences in composition, notably in the metal/hydrogen ratio, in stars which are extremely old. Some of the white dwarfs may be relics of the oldest stars, at the end of their evolutionary track. Most white dwarfs show lines only of hydrogen, but it is not yet known whether this means a very low metal content; some white dwarfs show only He, but a few show lines of Ca and Fe. (Incidentally, both the red giants and the white dwarfs seem to lose matter to interstellar space, so that it is not only by explosion, as in a nova or a supernova, that matter can be mixed back into space.) A large group of very old stars exists, which belong either to the central region or to the extended halo of our galaxy. They travel by the sun because of their large random space motions. Such high-velocity stars may still have energy sources, since they are intrinsically faint and have not burned up their hydrogen. They cannot have been formed recently, since they come from regions now free of gas. They normally show a low metal/hydrogen ratio, a reduction by perhaps a factor of two. The extreme members of this group are the high-velocity cool subdwarfs. In these, the abundance deficiency of the metals may reach a factor of twenty. Such stars can be relics of the earliest days of element and star formation, although it must be admitted that so far no star containing only hydrogen has ever been found.

It appears to us that a successful chain of reactions for synthesis in stars of elements up to Pb has now been found. Many types of stars are clearly now synthe-

sizing elements at least up to carbon and, in the S stars, up to the rare earths. The very oldest stars seem to show a deficiency of the elements heavier than He. Thus a substantial set of arguments exists for the possible continuing synthesis of the elements by a noncatastrophic process. We dare not assert that there are no serious objections to the theory, nor is the "beginning" some five billion years ago less mysterious whether the universe began as cold, simple hydrogen or as an expanding cloud of high-energy photons and neutrons.

In this general review we have not given detailed references to the work of others. Many of these physical processes were described by Bethe, G. and E. M. Burbidge, Cameron, Hoyle, and Salpeter. The astronomical evidence is from many sources, including the work of Aller, McKellar, Merrill, and Schwarzschild. Detailed references can be found in Fowler, Burbidge, and Burbidge, *Astrophysical Journal*, **122,** 271, 1955, and in J. L. Greenstein, *Publications of the Astronomical Society of the Pacific*, **68,** 1956.

*A COMPARISON OF THE HYDROGENASE ACTIVITIES OF DIFFERENT MICROÖRGANISMS**

By Alvin I. Krasna and D. Rittenberg

DEPARTMENT OF BIOCHEMISTRY, COLLEGE OF PHYSICIANS AND SURGEONS
COLUMBIA UNIVERSITY, NEW YORK, NEW YORK

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Introduction.—Many microörganisms contain an enzyme or system of enzymes, called "hydrogenase," which activates molecular hydrogen. Most methods for the assay of hydrogenase activity measure the rate of reduction of some compound by molecular hydrogen. Numerous compounds, both organic and inorganic, have been used.[1] The rate of hydrogen evolution from compounds such as reduced methyl viologen has also been used.[2] Also, hydrogenase can catalyze the exchange reaction between hydrogen and heavy water and the ortho-para conversion of hydrogen,[3] neither of which processes requires the presence of hydrogen donors or acceptors. The rate of reduction of an acceptor by hydrogen is not necessarily a direct measure of the activation of hydrogen, since other enzyme systems or electron carriers may be involved in the utilization of the activated hydrogen for the chemical reduction. This has been clearly demonstrated by many authors. Hoberman and Rittenberg[4] found that dilute suspensions of *Bacillus lactis aerogenes* or of *Proteus vulgaris* failed to reduce methylene blue but catalyzed the exchange reaction. They also found that 2 per cent urethan inhibited the reduction of methylene blue by heavy suspensions of these cells but had no effect on the exchange reaction. Shug *et al.*[5] have shown that molybdenum is required for the reduction of cytochrome c by the hydrogenase of *Clostridium pasteuranium* but is not required for the reduction of methylene blue. Curtis and Ordal[6] found that the hydrogenase of *Micrococcus aerogenes*, though capable of catalyzing the exchange reaction and the reduction of benzyl viologen, does not catalyze the reduction of methylene blue. Whiteley and Ordal[7] found that extracts of *M. lactilyticus* which are unable to

reduce methylene blue can reduce benzyl viologen. We therefore suggest that the term "hydrogenase" be limited to that enzyme which reversibly activates molecular hydrogen.

In the present report a comparative study is made of the rates of the exchange reaction and of the reduction of various acceptors by different microörganisms.

Experimental.—All experiments were carried out at 37° C. and pH 6.7 on whole-cell suspensions which had been washed twice with distilled H_2O and suspended in distilled H_2O.

The procedure used for determining the rate of the exchange reaction has been previously described.[3] Twenty-five milligrams of sodium dithionite were routinely added to the reaction vessel just before evacuation to remove traces of oxygen.[8]

Uptake and production of gas were measured in the conventional manner in the Warburg apparatus. The main compartment of the Warburg flask contained 0.2 mg. of crystalline bovine albumin, the appropriate amount of cell suspension, and 0.15 *M* phosphate buffer to make the volume 2.6 ml. The side arm contained 10 μM of the compound to be reduced in a volume of 0.2 ml. The center well contained 0.2 ml. of Fieser's solution[9] and a strip of folded filter paper to absorb traces of oxygen. The flasks were gassed with H_2 (for H_2 uptake) or N_2 (for H_2 production) for 10 minutes and then shaken for 1 hour before addition of the contents of the side arm. This lengthy equilibration was necessary in order to remove the last traces of oxygen from the system. Readings were then taken every minute or two until the H_2 uptake was no longer linear. For measurement of H_2 production from reduced benzyl viologen, 6 mg. (a threefold excess) of sodium dithionite were added to the 10 μM of dye in the side arm. The dye at this stage was yellow in color and turned violet when added to the main compartment.

The amount of the cell suspension used in each experiment was such that there was proportionality between it and the rate of the reaction.[3]

Results.—The complexity of the transfer process of hydrogen to an acceptor system is illustrated by the qualitative differences in reducing power of the hydrogenase of different organisms. Table 1 summarizes the ability of *Desulfovibrio desulfuricans*, *P. vulgaris*, and *Rhodospirillum rubrum* to reduce nitrate, nitrite, hydroxylamine, and nitroprusside.

TABLE 1

REDUCTION OF SOME NITROGEN COMPOUNDS BY THE HYDROGENASE OF DIFFERENT ORGANISMS

Organism	Exchange	Methylene Blue	NO_3^-	NO_2^-	NH_2OH	Nitroprusside
D. desulfuricans	+	+	−	+	+	+
P. vulgaris	+	+	+	−	+	+
R. rubrum	+	+	−	−	−	−

In all cases where there was no reduction, addition of catalytic amounts of methylene blue or benzyl viologen caused no reduction. A boiled extract of *D. desulfuricans* did not enable *P. vulgaris* to reduce NO_2^-, and a boiled extract of *P. vulgaris* did not enable *D. desulfuricans* to reduce NO_3^-. When NO_2^- or NH_2OH was reduced, an equivalent quantity of ammonia was formed, and the gas uptake was consistent with this reduction to ammonia.

Both *P. vulgaris* and *R. rubrum* reduce Janus green ($E_0' = -0.256$) with the

uptake of 2 moles and 1 mole of H_2, respectively. *Proteus vulgaris* reduces safranin Y ($E_0' = -0.289$) but not neutral red ($E_0' = -0.340$). *Rhodospirillum rubrum* reduces neither dye. *Proteus vulgaris* reduces benzyl viologen ($E_0' = -0.359$) faster than *R. rubrum*, and the latter reduces azocarmine G ($E_0' = -0.280$) faster than *P. vulgaris*.

Each of the three organisms can catalyze the exchange reaction. However, they are not equally capable of reducing various substrates. Clearly the reduction of the substrate is determined not solely by its oxido-reduction potential but also by the requirement for heat-labile factors or additional enzyme systems which are not needed for the exchange reaction. Indeed, enzyme systems are known which can effect the reduction of nitrate, nitrite, and hydroxylamine by reduced coenzymes.[10]

Every organism which we have tried which can reduce methylene blue catalyzes the exchange reaction. The rates of reduction of methylene blue and benzyl viologen and their relationship to the rates of the exchange reaction are shown in Table 2. There is a wide range in both the exchange activity and the rate of reduction of methylene blue as measured by Q_{H_2} and a similarly wide spread in the ratio of these two activities.

TABLE 2

COMPARISON OF EXCHANGE AND REDUCING ACTIVITIES OF DIFFERENT ORGANISMS

ORGANISM	Q_{H_2}*		RATIO		
	Exchange	Methylene Blue	Methylene Blue/ Exchange†	Benzyl Viologen/ Exchange†	Reduced Benzyl Viologen/ Exchange‡
E. coli	41,000	24,000	0.60	0.069	0.011
P. vulgaris	6,000	4,500	0.75	0.039	0.064
D. desulfuricans	40,000	58,000	1.45	0.45	0.077
C. pasteuranium	2,500	9,200	3.6	1.57	0
Acetobacter peroxydans§	170	670	3.9	...	...
R. rubrum	24‖	153	6.4	0.28	0
Azotobacter vinelandii	260	7,500	28.5	...	...

* Q_{H_2} is the microliters of hydrogen activated or used for reduction per milligram of nitrogen per hour.
† These columns give the ratio of the rate of hydrogen uptake by the indicated dye to the rate of the exchange reaction.
‡ This column gives the ratio of the rate of hydrogen production from reduced benzyl viologen to the rate of the exchange reaction.
§ We are indebted to Dr. S. Tanenbaum for supplying these cells.
‖ Addition of a boiled extract of either *P. vulgaris* or *D. desulfuricans* to *R. rubrum* or *Acetobacter peroxydans* had no activating effect on the exchange reaction.

The rates of the ortho-para hydrogen conversion for *P. vulgaris* and *R. rubrum*, expressed as Q_{H_2}, were measured and are 10,000 and 25, respectively.

The exchange reaction, the ortho-para conversion, and the reduction of the various substrates and dyes can be explained as the result of the following reactions:

$$E + H_2 \underset{k_2}{\overset{k_1}{\rightleftharpoons}} E{:}H^- + H^+, \tag{1}$$

$$E{:}H^- + S + H^+ \xrightarrow{k_3} E + SH_2. \tag{2}$$

These reactions require that the ortho-para conversion and the exchange reaction occur by the same mechanism, i.e., by the interaction of the gas phase with the water through reaction (1).[3] In those cases in which the data exist, the rates of the ortho-para conversion and the exchange reaction are the same. The different

ratios of the methylene blue reducing activity to the exchange reaction are probably a consequence of the differences in the reaction rates in different organisms of the reactions shown above.

Before hydrogen can exchange with water or be used in the chemical reduction of any substrate, it must be split by the forward step of reaction (1). The reaction with the rate constant k_1 is common to all organisms which activate molecular hydrogen. Depending, however, on whether k_3 is greater or smaller than k_2, the reduction of methylene blue can be faster or slower than the exchange reaction (or ortho-para conversion). If a cofactor is necessary for reaction (2), then it is possible that those organisms which display a low ratio of methylene blue to exchange are deficient in that factor.

It is possible that the value of k_2 varies from organism to organism, the values of k_1 and k_3 remaining constant. As k_2 becomes smaller, the rate of the exchange reaction(or ortho-para conversion) relative to the rate of chemical reduction decreases. The value of E_0' for the enzyme system will also differ among the various organisms, since it is determined by the ratio k_1/k_2. This suggests that the E_0' of the hydrogenase of *Azotobacter vinelandii* is more positive than that of the other organisms tested.

The same considerations apply to the rates of reduction of benzyl viologen and of hydrogen production from reduced benzyl viologen (see Table 2).

The ratio of the different activities to the exchange reaction does not follow a pattern; it is, in fact, quite haphazard. This suggests that some of the organisms might be lacking in the necessary factors or enzymes to reduce methylene blue or benzyl viologen, and therefore a systematic variation in the ratio of the different activities among different organisms is not to be expected. Indeed, the value of k_2 may differ for each substrate.

Similar results on the rates of reduction of dyes have been obtained by Peck, San Pietro, and Gest.[11] However, we find exchange activity in every organism which shows chemical reduction of methylene blue. The mechanism these authors propose is not consistent with all the available data. According to their mechanism, *R. rubrum* should catalyze the ortho-para conversion more rapidly than the exchange reaction. This is not the case. Second, the suggestion of these authors that the H_2 molecule is split to yield two hydrogen ions is inconsistent with our finding that *P. vulgaris* does not catalyze the ortho-para conversion when the liquid phase is D_2O[3] and that the first species to appear in the gas phase is HD and not D_2.[12]

The difficulties associated with methods which rely on the reduction of a dye for the determination of hydrogenase activity are illustrated by the effect of methylene blue on the exchange reaction of *P. vulgaris*. In this system the methylene blue not only competes for the activated hydrogen but also inhibits the enzyme system which deoxygenates hydrogenase. This deoxygenation is an essential step for the development of hydrogenase activity.[8]

The effect of methylene blue on the exchange reaction in *P. vulgaris* and *Escherichia coli* is shown in Table 3. In some of these experiments the methylene blue was added to the main chamber of the flask together with the cell suspension, the flask evacuated, filled with H_2, and then shaken. The presence of methylene blue caused an increase in the lag phase of the exchange reaction (see col. 4 of Table 3).

This increased lag phase is equal to the time required to reduce the methylene blue as evidenced by decolorization. The values in column 6 of Table 3 give the time required to reduce methylene blue when it was added to the cells from a side arm

TABLE 3

EFFECT OF METHYLENE BLUE ON THE EXCHANGE REACTION

Organism (1)	Methylene Blue Added* (μM) (2)	Lag phase for Exchange Reaction (Min.) (3)	Increased Lag Phase Due to Addition of Methylene Blue† (Min.) (4)	Rate of Exchange after Lag Phase (μl H_2/Hr) (5)	Time Required to Reduce Methylene Blue‡ (Min.) (6)
E. coli	0	37	...	1,830	..
	5	42	5	1,650	6
	10	47	10	1,770	12
P. vulgaris	0	8	...	1,690	..
	5	75	67	1,410	5
	10	110	102	1,710	10

* The methylene blue was added to the cells before filling with hydrogen.
† This is also the time required to reduce the methylene blue as indicated by decolorization.
‡ The methylene blue was added after the cells had been shaken under hydrogen for an hour.

after the cells had been shaken under hydrogen for an hour. With *E. coli* these two times were equal. With *P. vulgaris* the lag phase (and time required to reduce methylene blue) when the methylene blue was added to the cells before incubation under hydrogen was more than ten times that required to reduce methylene blue when it was added to the cells after incubation for an hour under hydrogen. When the air in the system is replaced by hydrogen, there still remain traces of oxygen which inhibit hydrogenase.[8] This oxygen is slowly consumed by the cells upon incubation. It would seem that methylene blue inhibits those systems in *P. vulgaris* which utilize oxygen. This increases the lag phase of the exchange reaction and the time required to reduce methylene blue. This inhibition is not observed with *E. coli*.

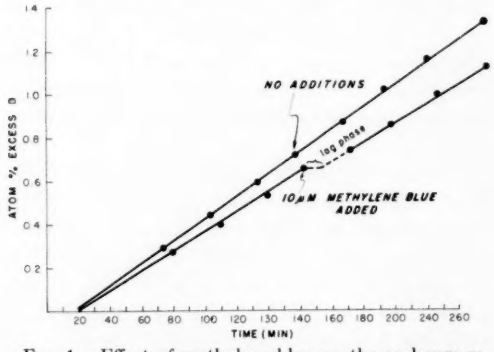

FIG. 1.—Effect of methylene blue on the exchange reaction. Methylene blue in a volume of 0.2 ml. was added to the flask at 163 minutes, as indicated on the curve. Addition of 5 μM of methylene blue produced a lag phase of 7 minutes, and 10 μM of methylene blue produced a lag phase of 11 minutes. Two-tenths of a milliliter of distilled water was added to the control flask at the same time that methylene blue was added to the other flask. The dotted lines do not represent the actual course of the exchange reaction but are constructed merely to estimate the lag phase.

If methylene blue inhibits the oxygen-removing systems in *P. vulgaris*, addition of methylene blue to *P. vulgaris* after the oxygen is removed should produce a lag phase corresponding to the time required to reduce the methylene blue (under identical conditions) and no longer. This is the case (see Fig. 1). In this experiment the methylene blue was placed in a side arm and was added to the main vessel after the exchange reaction had progressed for 163 minutes. At this time, when all the oxygen originally present had been reduced, the lag phase was equal to the time

required to reduce the methylene blue. It is clear that the long lag phase observed when methylene blue was present before all the oxygen was removed is due to an inhibition by methylene blue of the systems of the cell which consume oxygen.

Summary.—The rates of the exchange reaction and of the reduction of various dyes and other acceptors have been compared in a number of different microorganisms. While the activation of hydrogen, as measured by the exchange reaction, appears to be relatively simple, the utilization of the activated hydrogen for chemical reduction requires cofactors or additional enzyme systems.

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*GROWTH RESPONSE OF SINGLE-GENE DWARF MUTANTS IN MAIZE TO GIBBERELLIC ACID**

BY BERNARD O. PHINNEY

DEPARTMENT OF BOTANY, UNIVERSITY OF CALIFORNIA, LOS ANGELES

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Gibberellic acid, $C_{19}H_{22}O_6$, was first isolated by Cross[1] from the *Fusarium moniliforme* Sheldon stage of *Gibberella fujikuroi* (Saw.) Wr. It is a tetracyclic dihydroxy lactonic acid, identical to gibberellin X, reported by Stodola *et al.*,[2] and distinct from gibberellin A, isolated by Yabuta and Hayashi.[3] Both gibberellic acid and gibberellin A markedly stimulate stem and leaf elongation in a number of higher plants.[3, 4] Of particular interest to geneticists is the report of Brian and Hemming[4] that slow-growing varieties of peas (*Pisum*) will respond more than fast-growing varieties to applications of gibberellic acid. They also reported a similar differential growth response to applications of gibberellic acid between tall and dwarf varieties of broad bean (*Vicia*) and French bean (*Phaseolus*), concluding that

the causes for dwarfism in these plants are probably the same as those acting in peas.

In the present study, it is shown that normal growth can be induced in dwarf mutants of maize by the addition of gibberellic acid. Certain other mutants for dwarfism in maize show no growth response or only a very slight response to this compound.

MATERIALS AND METHODS

Of the six mutants segregating from the stocks[5] used in these experiments, *anther ear-1*, *dwarf-(5232)*, *dwarf-1*, *dwarf-(8201)*, and *dwarf-(4963)* are nonallelic to each other and are controlled by simple recessive genes. The sixth mutant, *dominant-dwarf*, is a simple dominant of unknown linkage. All mutants appear as dwarfs from the seedling stage to maturity, mature mutants being less than half the height of mature normals.

Gibberellic acid was prepared as aqueous stock solutions from two samples.[6] The first sample consisted of an equal mixture of gibberellic acid and gibberellin A. The second sample was pure gibberellic acid, $[\alpha]_D^{25}$, = 92°. Gibberellic acid was applied by placing a volume of known concentration on the surface of the first unfolding leaf of 8-day-old seedlings or on the upper leaves of older plants. In each treatment gibberellic acid was applied to at least six normal and six mutant plants. All experiments were replicated at least twice.

EXPERIMENTAL

Response of Different Mutants to Gibberellic Acid: Experiment 1.—Normal and mutant seedlings from each of the six stocks were given single applications of 10 μg. of gibberellic acid (pure sample) per plant at the time of emergence of the first seedling leaf from the coleoptile. The growth response of treated normal seedlings over that of nontreated normal seedlings was slight and was not evident until several days after application. However, the mutants *anther ear-1*, *dwarf-(5232)*, *dwarf-1*, and *dwarf-(8201)* showed a growth response that was evident within 24 hours following treatment. These four mutants continued to respond, so that at 2 weeks of age they were very similar in height to treated normals of the same age. However, without further applications of gibberellic acid, they slowly returned to the dwarf habit of growth. Normal and *dwarf-1* seedlings, with and without treatment, are shown in Figure 1. In marked contrast to the response of the above four mutants, *dominant-dwarf* seedlings showed no growth response and *dwarf-(4963)* seedlings only a very slight growth response.

These mutants, *dominant-dwarf* and *dwarf-(4963)*, were tested further by repeat runs. Ten μg. of gibberellic acid (pure sample) per plant was added to normal and mutant seedlings at the time of emergence of the first leaf, and supplementary additions of 5 μg. per plant were given at daily intervals for 5 days. With this amount applied over the period of 5 days, normal seedlings showed unusual elongation of leaf blades as well as of leaf sheaths. *Dominant-dwarf* seedlings showed no growth response. *Dwarf-(4963)* seedlings showed some growth response, but they still retained the dwarf growth habit, remaining less than half the height of nontreated normal seedlings.

In other tests these two mutants were given a single application of 100 μg. of

gibberellic acid (pure sample) per plant. Again *dominant-dwarf* seedlings showed no response and *dwarf-(4963)* seedlings only a limited response.

Sensitivity of Response of Mutant Seedlings to Gibberellic Acid: Experiment 2.—The four stocks segregating *anther ear-1*, *dwarf-(5232)*, *dwarf-1*, and *dwarf-(8201)* were used in this experiment. Normal and mutant seedlings were given single applications of gibberellic acid (pure sample) at the time of emergence of the first leaf. The amounts used ranged from 0.01 to 20 μg. per plant. Normal seedlings showed no observable growth response with amounts less than 0.5 μg. per plant. Mutant seedlings from each of the four stocks showed a slight but noticeable growth response with as little as 0.01 μg. per plant. Both normal and mutant seedlings

FIG. 1.—Response of normal and *dwarf-1* seedlings to a single application of 10 μg. of gibberellic acid (pure sample) per plant. Treatments given at the time of emergence of the first leaf. Plants shown are 2 weeks old.

responded to the addition of amounts greater than 1.0 μg. per plant, the growth response of the mutant seedlings being much greater than that of the normal seedlings. Mutant seedlings ultimately reached the same height as treated normal seedlings with 10, 15, and 20 μg. of gibberellic acid per plant. With only single applications, all mutant plants reverted to the dwarf habit of growth. In the range studied, the degree of response was related to the dosage: the greater the dosage, the greater the response.

Response of Mutant Plants to a Continuous Supply of Gibberellic Acid. Experiment 3.—The four mutants *anther ear-1*, *dwarf-(5232)*, *dwarf-1*, and *dwarf-(8201)* were tested for their growth response to multiple applications of gibberellic acid. Nor-

mal and mutant seedlings were given 10 μg. of gibberellic acid (mixture) per plant at the time of emergence of the first leaf. Additional amounts, totaling 60 μg. per plant, were supplied to the uppermost leaves at 2- or 3-day intervals for 8 weeks. All seedlings showed an initial response as described in the previous experiments. Normal plants showed only a slight response in later stages of growth. The four mutants continued to respond, becoming very similar to treated normal plants in size and shape. Normal and *dwarf-1* plants, with and without treatment, are shown in Figure 2.

FIG. 2.—Response of normal and *dwarf-1* plants to multiple applications of gibberellic acid (mixture), totaling 60 μg. per plant. Treatments initiated at the time of emergence of the first leaf and continued at 2–3 day intervals. Plants shown are 9 weeks old.

DISCUSSION

Evidence for the conversion of a mutant phenotype to a normal phenotype by the addition of a particular compound has been well documented in microörganisms, especially in *Neurospora*.[7] These contributions have led to many significant advances in the study of gene action.[8] In contrast to the vast amount of information of this nature for microörganisms, there are few reports for higher plants where mutants respond to a given compound to reach a normal phenotype. Langridge[9] has reported a case in the crucifer, *Arabidopsis thaliana* (L.) Hayn., where a simple recessive mutant responded to thiamine to give normal growth.

Brian's work[4] on the growth response of dwarf varieties of beans and peas to gibberellic acid suggests that this response is probably under genetic control. The four mutants of maize *anther ear-1*, *dwarf-(5232)*, *dwarf-1*, and *dwarf-(8201)* are clear examples of cases in which the ability to respond to gibberellic acid is under the control of single genes. The response of these four mutants and the lack of response of the two mutants *dominant-dwarf* and *dwarf-(4963)* indicate that the reason for dwarfism can vary, depending on the particular gene controlling the expression of the dwarfing character. Studies are being made on the mechanism of the gibberellic acid response in these mutants.

SUMMARY

1. The growth response of six single-gene mutants in maize to gibberellic acid is reported. Four of these, *anther ear-1*, *dwarf-(5232)*, *dwarf-1*, and *dwarf-(8201)*, responded by normal growth. The fifth mutant, *dominant-dwarf*, did not respond and the sixth mutant, *dwarf-(4963)*, showed only a slight response.

2. The sensitivity of response to gibberellic acid and the requirement of a continuous supply of gibberellic acid to maintain normal growth for four of the mutants are reported.

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*THE q LOCUS OF NEUROSPORA CRASSA**

BY PATRICIA ST. LAWRENCE[†]

DEPARTMENT OF MICROBIOLOGY, YALE UNIVERSITY, NEW HAVEN, CONNECTICUT

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Investigations with several organisms have demonstrated that crosses of individuals carrying mutant alleles may give rise to rare nonmutant progeny. In a number of instances[1] the event which produced the exceptional offspring was shown to involve crossing over within a small chromosomal segment. In *Neurospora crassa*, Bonner[2] has described the occurrence of rare nonmutant progeny in crosses of niacin-requiring mutants, which he has designated "*q* mutants." These mutants do not form niacin-independent heterocaryons with one another, and there is evidence that all of them are blocked in the same step in niacin synthesis.[2] Apparently, no similar cases have been reported in which both a genetic study and a biochemical

analysis of the metabolic alterations in the mutants were performed. The *q* mutants therefore offered unusual advantages for a study of the emergence of non-mutant progeny. The origin of the *q* mutants used in this investigation is given in the accompanying table.

Symbol Used in This Publication	Isolation Number	Mutagen Used	Reference
q1	3416	X-ray	1
q2	1413	Ultraviolet	2
q3	39113	Ultraviolet	1
q4	Y31873	Nitrogen mustard	3

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Reversion Behavior.—Investigations of the reversion rates of the *q* mutants have shown them to be exceedingly stable in the vegetative state. Conidia of *q1* stocks have shown spontaneous reversions of the order of 0.3–0.5 per 10^8 macroconidia. No spontaneous reversions have been recovered from macroconidia of the other *q* strains; in the case of *q4*, it was not even possible to induce reversions with ultraviolet or X-rays.

Linkage Behavior.—Since all four of the *q* mutants were originally induced in different stocks and outcrossed during the process of selection, the available stocks would be expected to differ in their genetic background. Therefore, an extensive analysis of the relationships of the four *q* mutants to certain linked loci was undertaken. These experiments were also designed to provide stocks of the *q* mutants carrying markers suitable for analysis of niacin-independent progeny which might occur in crosses.

The *q* mutants are located in linkage group I, and Pittenger[3] has shown that *q1* is closely linked to *lysine-3*[4] (the symbol used here is *lys*). In addition to *lys*, the linked markers *albino-2*[4] (*al*) and *osmotic* (*os*) were used.

The sequence of markers from the centromere is as follows: *al, lys, os*. Analysis of ascospores isolated at random indicates that there is approximately 15 per cent recombination between *al* and *lys* and approximately 20 per cent recombination between *lys* and *os*. These values, derived from a number of crosses, were here taken as standard for the genetic analysis of the *q* mutants.

The *q* mutants are located between *lys* and *os* and show approximately 0.5–1 per cent recombination with *lys*. Stocks of both *q1* and *q2* have been obtained that show the standard recombination values given above when crossed to niacin-independent stocks carrying various combinations of the markers *al, lys*, and *os*. However, in both *q3* and *q4*, crossing over in the interval from *al* to *lys* was significantly reduced; all strains of these mutants derived from a number of crosses and carrying various markers have shown this reduction, as have crosses of *q3* by *q3*, and *q4* by *q4*. Tests with *q3* suggest that reduction in recombination in the interval between *al* and *lys* is inseparable from the mutant character of *q3*. At present it is not known whether a similar situation obtains in the case of *q4*.

Mutant *q4* has a distinctive characteristic: in contrast to the other *q* mutants, 1–2 per cent of the progeny isolated from crosses of *q4* to various stocks do not grow well on any media tested. Therefore, it was not possible to characterize such isolates

with respect to the markers segregating in the cross. Thus, among approximately 1,000 ascospores isolated at random from crosses of *lys* by *q4*, no strain carrying both *q4* and *lys* was recognized, although the reciprocal recombinant was obtained.

Isolation of Niacin-independent Progeny.—In order to examine the possibility that niacin-independent colonies obtained from crosses between the *q* mutants arise as the result of crossing over, a number of strains were prepared with suitable combinations of markers. Crosses were then made between strains carrying the same or different *q* mutants. For convenience the former type of cross will be referred to as a "selfing." The crosses were set up in a reciprocal manner: a particular combination of markers was introduced by one parent in one series and by the other parent in a second series. Unfortunately, reciprocal crosses could not be performed with *q4* because lysine-requiring strains of this mutant were not recovered.

In these crosses it was necessary to determine whether the markers used affected the frequency of recovery of niacin-independent colonies. Accordingly, crosses of *q* mutants carrying markers were compared with analogous crosses of *q* mutants without markers; at least four separate stocks each of *q1*, *q2*, and *q3* and three of *q4* were used.

To obtain niacin-independent colonies, mature ascospores from these two types of crosses were collected in distilled water, heat-shocked in a sorbose-containing medium supplemented with any required growth factors except for niacin, plated in the same medium, and incubated at 30° C. The total number of ascospores plated was ascertained by direct count. Germination was determined by heat-shocking and plating ascospores in suitably supplemented medium containing niacin, and counting the germinated spores after incubation. In almost all crosses, 90 per cent or more of the ascospores germinated.

The results obtained indicated that the presence or absence of segregating markers did not significantly influence either the per cent germination or the frequency of niacin-independent colonies recovered. Crosses between any two different *q* mutants gave niacin-independent colonies, although only three such colonies were recovered from crosses of *q1* and *q3*. No niacin-independent colonies were recovered from any of the four possible selfings; at least 10^5 ascospores were examined for each selfing.

The frequency with which niacin-independent colonies were obtained is given in the second column of Table 1. It can be seen that the frequency of occurrence of such colonies in reciprocal crosses is approximately constant. It therefore appears

TABLE 1

DISTRIBUTION OF MARKERS IN NIACIN-INDEPENDENT ISOLATES FROM CROSSES BETWEEN *q* MUTANTS

MUTANTS CROSSED	NO. OF NIACIN-INDEPENDENT ISOLATES/10^4 VIABLE ASCOSPORES	NO. OF ISOLATES WITH NO CROSSOVER BETWEEN *lys* AND *os*			NO. OF ISOLATES WITH A CROSSOVER BETWEEN *lys* AND *os*			TOTAL NO. OF ISOLATES ANALYZED	RATIO OF *lys*/*lys*$^+$ RECOVERED
		lys q / *os*			*lys q* / *os*				
		+ *os*	*lys* +	Total	*lys os*	++	Total		
q1 + *os* × *q2 lys* +	4.3–6.9	25	14	39	18	15	33	72	0.80
q2 + *os* × *q1 lys* +	4.1–7.5	8	13	21	7	17	24	45	0.84
q2 + *os* × *q3 lys* +	4.4–6.5	18	18	36	9	7	16	52	1.18
q3 + *os* × *q2 lys* +	4.9–6.3	10	4	14	5	2	7	21	0.75
q4 + *os* × *q1 lys* +	0.8–1.3	1	16	17	6	19	25	42	1.10
q4 + *os* × *q2 lys* +	2.1–2.5	2	13	15	13	3	16	31	5.20
q4 + *os* × *q3 lys* +	0.4–0.8	2	17	19	9	6	15	34	3.25

reasonable to conclude that the recovery of niacin-independent progeny results from events at the *q* locus in the process of crossing the different mutants.

Analysis of Niacin-independent Progeny.—For a study of the distribution of the segregating markers in the niacin-independent progeny, a sample of approximately 20–70 colonies from each type of marked cross was examined. Because of the possibility that some or all of the niacin-independent progeny were pseudo-wilds,[3, 5] each of the colonies isolated was crossed to wild type. In each case, asci consisting entirely of niacin-independent spores were obtained. Therefore, these asci presumably were not derived from a pseudo-wild parent or from a parent carrying a randomly segregating suppressor. Such niacin-independent asci were used for the determination of the distribution of markers.

The results obtained are presented in Table 1. The data have been grouped in two classes: one class represents the recovery of parental chromatids in which no crossovers in the interval from *lys* to *os* were detected, and the second class represents chromatids in which a crossover in this interval was found. Table 1 provides the following evidence regarding the question whether or not the occurrence of niacin-independence is related to crossing over:

a) If the *q* mutants are arranged along the chromosome in a linear manner and if niacin-independence is related to crossing over in a region between the *q* mutants, the majority of the niacin-independent isolates would be expected to occur in the crossover class. However, it is seen that in general there are approximately equal numbers of niacin-independent isolates in the crossover and in the noncrossover classes. Crosses of *q2* to *q3* appear exceptional in that niacin-independent colonies were recovered somewhat more frequently in the noncrossover class.

b) Table 1 shows that both of the two possible recombinant classes of *lys* and *os* are represented in the niacin-independent progeny from all crosses. However, if niacin-independence arises as the result of crossing over, the recovery of only one of the recombinant classes would be expected.

On the assumption that the class in which no crossovers were detected between *lys* and *os* represents recovery of an even number of crossovers of the two-strand type, the niacin-independent isolates would again be expected to carry one of the two possible arrangements of the *lys* and *os* markers. Table 1 shows that in general both arrangements are well represented, although in crosses involving *q4* one of the arrangements predominates.

c) If niacin-independent isolates arise as the result of crossing over between *q* mutants, the vast majority of these isolates should carry one of the two possible alleles of *lys* (because of the close linkage between *q* and *lys*). Furthermore, the reciprocal crosses should exhibit a reciprocal relationship with respect to the *lys* allele carried by the niacin-independent isolates. In the sixth column of Table 1 is presented the ratio of niacin-independent isolates that required *lys* to those that did not. In general this ratio approximates unity, although in crosses of *q4* by *q2* or *q3*, isolates carrying *lys* are in moderate excess.

The results discussed in *a*, *b*, and *c* above fail to provide evidence for the occurrence of niacin-independence as the result of crossing over in the usual sense. If crossing over were to account for the results obtained, the number of multiple crossovers that would be required in most cases is equal to, or exceeds, that of the single crossovers. On the other hand, the emergence of niacin-independent isolates

does not seem entirely unrelated to crossing over. Thus it is of interest that recombination between *lys* and *os* is in general twice as frequent in the niacin-independent as in the niacin-requiring isolates from any cross. A similar increase in recombination has been reported in investigations of other series of biochemically similar mutants.[6, 7]

In the various crosses given in Table 1, the marker classes obtained for any pair of *q* mutants seem characteristic and not random. This nonrandomness is particularly clear in crosses involving *q4*. Table 1 reveals that the marker combination brought into a given cross by *q4* is present in very low frequency in the niacin-independent progeny. Since the same marker combination appears in high frequency if introduced by a mutant other than *q4*, it would appear that the marker combination as such was not selected against. It seems reasonable to conclude that the distribution patterns of the markers in niacin-independent isolates depend on the individual *q* mutants and their interaction in crosses.

Possible Origin of Niacin-independence.—Since, in the crosses examined, the occurrence of niacin-independence does not appear to be caused by crossing over, other mechanisms must be considered. A similar problem was encountered by Mitchell[6] in her elegant and convincing analysis of the *pdx* mutants of *Neurospora* and by Lindegren[8] in his study of yeast mutants. Both authors have considered the possibility of a gene-to-gene conversion similar to that proposed by Winkler[9] some time ago. The appearance of niacin-independence in the present experiments may also be said to represent gene conversions in a broad sense or may be thought of as reflecting a kind of mutation.

Among possible mechanisms that could account for the present observations, one, suggested by the incisive studies of McClintock[10] on mutation in maize, seems especially worthy of consideration. She has shown that the insertion of chromosomal material into, or near to, the locus of a number of known mutants may cause a mutant phenotype to appear. In some cases the insertion of material leads also to changes in linkage relationships.[11] Removal of the inserted material may result in return to the original phenotype.

It may therefore well be that the niacin requirement of the *q* mutant is occasioned by the insertion of material into, or near, the functional area of the chromosome concerned with one step in niacin synthesis. Some support for this "insertion hypothesis" is provided by the present findings that at least two of the *q* mutants, *q3* and *q4*, affect linkage relationships in neighboring areas of the chromosome. It would appear likely that these mutants reflect structural alterations of the chromosomal material rather than point mutations. Mutants *q1* and *q2* may also be the result of such structural alterations, although they behave like point mutations within the relatively low sensitivity of the methods used. Occasional removal of the inserted chromosomal material in the course of crosses between *q* mutants could readily account for the appearance of niacin-independence.

Summary.—A genetic study of four biochemically similar niacin-requiring strains of *N. crassa* has been undertaken. The mutants involved (*q* mutants) have been characterized as to linkage relationships. Only one of the mutants gives rise to spontaneous reversions (at an infrequent rate) in vegetative culture.

Crosses between different *q* mutants yielded (rare) niacin-independent progeny, whereas "selfings" did not. Niacin-independent progeny were recovered whether or

not a crossover was detected in a marked region including the *q* locus, and regardless of the direction of any such crossovers. For these reasons, and from a consideration of the relative frequency of niacin-independence in the various marker classes obtained, it was concluded that niacin-independence does not seem to be the result of crossing over in the usual sense. Nevertheless, increased crossing over in the marked region appeared to be associated with the occurrence of niacin-independence.

The possibility has been discussed that various characteristics of the *q* mutants are occasioned by the insertion of chromosomal material at or near the region of the *q* locus.

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A CASE OF POLYPLOIDY IN DIPTERA

BY HARRISON D. STALKER*

DEPARTMENT OF ZOÖLOGY, WASHINGTON UNIVERSITY, ST. LOUIS, MISSOURI

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The repeated discovery of polyploidy in animals which show parthenogenesis, hermaphroditism, or vegetative reproduction and the rarity of its discovery in strictly bisexual forms have in a sense verified the prediction made by Muller[1] that the chromosome mechanism in many bisexual forms would tend to prevent the establishment of polyploid strains. Although apparent exceptions to this general prediction are known,[2] still it seems clear that Muller's prediction holds to a large extent. In female-producing (thelytokous) parthenogenetic species no problem of

unbalance between the sex chromosomes and autosomes exists, and one might expect to find polyploids in such forms.

In the Diptera, species regularly showing adult parthenogenesis are known in the families Lonchopteridae (1 species), Chamaemyiidae (1 species), and Agromyzidae (2 species). The author has studied the chromosomes of one of these forms, *Lonchoptera dubia* Curran, and found that, at least over the rather extensive range sampled, all specimens were diploid.[3] The present paper deals with a chromosomal study of two species of the family Chamaemyiidae.

In this family Dr. A. H. Sturtevant (personal communication) recognizes seven nearctic species belonging to the subgenus *Ochthiphila*. Normal-appearing males are known in at least five of these species, the clear exception being *O. polystigma* Meigen, for which the probability of obligatory parthenogenesis has been indicated.[4] *Ochthiphila polystigma* is reported from Europe, and in North America it extends northward to the Northwest Territories and Quebec and southward to Maryland, Missouri, New Mexico, and southern California. It may be collected by sweeping lawns, pastures, etc., and is apparently most abundant around quack grass (*Agropyron*), on which it is reported to breed.

In the summer of 1953, in the course of making a number of large collections of *O. polystigma* from the St. Louis area, the author found a single specimen which was clearly male or male-like. The external genitalia were slightly rotated and asymmetrical, and the gonads consisted of two small, unequal-sized sacs which contained masses of cells but no differentiated sperm. The general appearance of the individual was suggestive of a male-like intersex of the type found among the biparental progeny of triploid females in various *Drosophila* species. No satisfactory chromosome counts of the specimen could be obtained.

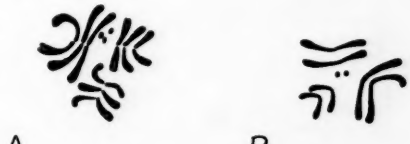

FIG. 1.—*A:* Triploid oögonial metaphase chromosomes from a specimen of *O. polystigma* taken at St. Louis, Missouri. *B:* Diploid oögonial metaphase chromosomes from a specimen of *O. geniculata* taken at Black Lake, Quebec.

The discovery of this possibly intersexual individual suggested that the species might be polyploid, with the abnormal specimen some sort of nondisjunctional product such as 2X 3A or 3X 4A. An examination of acetic-orcein smears of the ovaries of wild-caught females was begun in order to check this point. It was at once clear that the specimens from the St. Louis area were indeed polyploid, with three sizes of V-shaped major autosomes and a set of dots, each of the four kinds of chromosomes occurring in triplicate in metaphase, and indicating by the shape and size of the chromosomes and by the pattern of somatic pairing that the species was clearly triploid (see Fig. 1, *A*).

Since 1953, additional collections of wild *O. polystigma* females have been made from twenty-three localities. About one-fourth of the specimens so obtained gave unsatisfactory oögonial preparations, in most instances because of immaturity, but a total of 71 (see Table 1) were clearly triploid for all major autosomes and, in the majority of these cases, clearly triploid for the dot chromosome as well. Of the specimens giving unsatisfactory preparations (not entered in the table), none gave any indication of diploidy; however, one from Iowa City, Iowa, showed four dot-

shaped chromosomes and apparently had more than nine major autosomes, although the exact number could not be determined. The possibility exists that this specimen was actually tetraploid, but further material would be needed to establish the presence of a tetraploid race in the Iowa City area, especially since the other collections from surrounding areas showed clearly triploid individuals. Thus as yet no clear evidence exists for the presence of either diploids or tetraploids in *O. polystigma*, although their existence cannot be ruled out in other unstudied areas.

Since *O. polystigma* appears to be the only parthenogenetic species in its subgenus, the chromosomal constitution of other bisexual relatives is of some interest: discovery of polyploidy in these forms would suggest that it had arisen in *O. polystigma* prior to the development of parthenogenesis. Specimens of the bisexual species *O. geniculata* Zetterstedt were studied cytologically; Dr. A. H. Sturtevant kindly confirmed the specific determination.

TABLE 1

LOCALITIES FROM WHICH TRIPLOID FEMALES OF *O. polystigma* HAVE BEEN IDENTIFIED BY CYTOLOGICAL ANALYSIS

Locality	Triploids Identified	Locality	Triploids Identified
Colorado:		*New York:*	
Drake	2	Rochester	5
Loveland	1	Antwerp	5
Rocky Mt. Pk.	1	Potsdam	1
Iowa:		*Ontario:*	
Wheeler's Corners	4	Cobalt	3
Davis Corners	1	North Bay	2
Iowa City	1*	Pembroke	4
Michigan:		Sault Ste. Marie	1
Mackinac City	5	*Quebec:*	
Minnesota:		Portneuf	1
Fairbault	1	Bouchette	1
Missouri:		Granby	4
St. Louis	11	Black Lake	2
Wisconsin:		St. Édouard	10
St. Croix Falls	1	Rigaud	4

* An additional specimen from Iowa City clearly showed four dot-shaped chromosomes; the major autosomes although not entirely clear, suggested tetraploidy.

Three females of this species, coming from Black Lake, Quebec, Pembroke, Ontario, and Ray, Minnesota, were alike in showing three pairs of V-shaped chromosomes and a pair of dots in the oögonial metaphases. The somatic pairing and chromosome sizes clearly indicated diploidy (see Fig. 1, *B*). Thus there is at present no reason to believe that polyploidy arose in *O. polystigma* prior to the origin of parthenogenesis.

The Relationship between Polyploidy and Parthenogenesis.—It has not been possible to induce oviposition of *O. polystigma* in the laboratory, and nothing is certainly known of its chromosomal mechanism of parthenogenesis or of the evolutionary origin of its polyploidy. Although it is well known that occasional triploid individuals are produced in bisexual species (such as *Drosophila*, for instance) by fertilization of rare diploid eggs by haploid sperm, the resulting triploids are intersexual if male and are highly sterile, due to production of aneuploid gametes, if female. Thus permanent establishment of triploidy in such bisexual forms seems out of the question.

Suomalainen[5] states that constant anisopolyploidy (3n, 5n, etc.) of parthenogenetic forms can naturally arise only in species with apomictic (ameiotic) parthenogenesis. In fact, the majority if not all of the known anisopolyploid parthenogenetic animals are apomicts. Certainly the wide prevalence of triploidy in *O. polystigma* might suggest that this species is an apomict, since without meiosis the triploid condition would not lead to the production of inviable aneuploid progeny. But if it is indeed apomictic, the question of origin of the triploidy arises. Other workers[6] have generally assumed that anisopolyploidy in apomicts has arisen through hybridization of diploid apomictic females with diploid males from related bisexual species. If such hybridization could in fact lead to triploidy (assuming that the normally parthenogenetic eggs will form triploids when fertilized), or if hybridization of apomictic tetraploids to bisexual diploids would produce triploids, it still seems questionable whether the triploid females so produced would retain a genotype conferring a sufficiently high rate of parthenogenesis to compete successfully with their bisexual and parthenogenetic ancestors, since one-third of the triploid genotype is derived from the bisexual race or species. The above difficulties, although not insurmountable, are real and make the search for alternate hypotheses worth while.

An alternate pathway for the development of anisopolyploidy would be the following:

Diploid bisexual species
Diploid automictic parthenogenetic species
Anisopolyploid automictic parthenogenetic species
Anisopolyploid apomictic parthenogenetic species

This origin, involving passage through a temporary or permanent automictic phase, has the advantage of not requiring any production of anisopolyploidy via hybridization. Contrary to the opinions of Suomalainen[5] and White,[6] the author feels that certain types of automixis are just as suitable for maintenance of anisopolyploidy as is apomixis.

Any automictic mechanism which involves re-fusion of the meiotic products in such a way as to nullify the effects of the first reductional division would exactly reconstitute the original chromosomal constitution and should lead to no inviability due to aneuploidy, even in anisopolyploids. Two patterns of automictic parthenogenesis are known which would satisfy the above requirements. The first, which occurs in the diploid Psychid moth *Apterona helix*,[5] will not be discussed further, since, although it will support anisopolyploidy, it offers no clue as to its evolutionary origin.

A somewhat different automictic mechanism, which with certain refinements would permit constant anisopolyploidy to survive and to *arise*, is known in *Drosophila* and in *L. dubia*. In *D. parthenogenetica* occasional development of unfertilized eggs takes place with the formation of diploid or triploid females. In this species two meiotic divisions occur, resulting in the formation of four haploid meiotic products, which are arranged linearly across the egg.[7] The two outer nuclei come from one secondary oöcyte, the two inner from the other. Fusion of any two of the four nuclei results in the production of a diploid first-cleavage nucleus (fusion of three of the four results in production of a triploid cleavage nucleus and a triploid individual). In *D. parthenogenetica*, which is an essentially bisexual species,

with parthenogenesis poorly developed, the pattern of binucleate fusion is variable: about half the time, the two inner or two outer nuclei fuse; the rest of the time, fusion is between the two central nuclei. Modification of this system so that it involved regular fusion of the two *central* nuclei would immediately make it available for an anisopolyploid species, since such a fusion pattern would always reconstitute the original anisopolyploid chromosomal complement. This situation is indicated schematically below. In the diagram the letters *A* and *B* represent different (non-homologous) chromosomes. Thus the formula *AAABBB* represents an anisopolyploid (triploid) with two types of chromosomes. It will be noted that, with prereduction of centromeres, re-fusion of the complementary central nuclei will always reconstitute the original triploid condition.

It should be pointed out that while in *Drosophila* the binucleate fusion pattern appears to be random, in other forms, such as the Coccid *Lecanium hesperidum*, it regularly occurs between two terminal nuclei;[5] and in the fly *L. dubia* there is indirect evidence that fusion normally occurs between central nuclei. Thus the assumption of evolutionary restriction of the nuclear fusion pattern is not an unreasonable one. Even in a species in which there is regularly post reduction of centromeres, a modification of the above scheme, involving regular fusion of the two inner or two outer nuclei rather than of the central ones, would still support stable anisopolyploidy.

The mechanism of automixis outlined above permits change from diploidy to triploidy very readily, since, in a parthenogenetic diploid strain, infrequent fusion of three rather than two second-division products would result in production of triploid females, and such females, receiving no genes from a bisexual strain, should suffer no reduction in their parthenogenetic ability and might be expected to compete successfully with their diploid parthenogenetic ancestors.

The author wishes to take the position that not all instances of anisopolyploidy in parthenogenetic animals have developed by the mechanism outlined above but that such an evolutionary pathway is available, especially in the case of the Diptera.

Summary.—An investigation of the nearctic parthenogenetic species *Ochthiphila polystigma* Meigen (Chamaemyiidae, Acalypteratae) indicates that, at least over a wide part of its range, only triploid females occur. The bisexual species *O. geniculata* is shown to be diploid. Despite the triploidy in *O. polystigma*, it appears that it could be either apomictic (ameiotic) or automictic (meiotic), since certain types of automixis would result in restoration of the euploidy which might be temporarily lost in the first meiotic division. Examples of such automixis are the types found in *Drosophila parthenogenetica*, and *Lonchoptera dubia*. Reasons are given for concluding that anisopolyploidy (3n, 5n, etc.) in this and other species must have appeared subsequent to the origin of parthenogenesis and may have arisen in an automictic parthenogenetic phase.

It is a real pleasure to acknowledge the generous help of Dr. A. H. Sturtevant in connection with this work. The author has drawn freely on his unpublished notes regarding the taxonomy and range of the *Ochthiphila* species and has also benefited from personal communications regarding the possibility of polyploidy in *O. polystigma*.

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*ON COVERING A CIRCLE BY RANDOMLY PLACED ARCS**

BY ARYEH DVORETZKY

HEBREW UNIVERSITY OF JERUSALEM AND COLUMBIA UNIVERSITY, NEW YORK

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I

Let C denote a circle of unit circumference, and let a_i ($i = 1, 2, \ldots$) be a sequence of positive numbers smaller than 1. Let A_i be arcs of length a_i on C, and assume that the centers of these arcs are uniformly and independently distributed on C. If x is any given point on C, then[1] $P\{x \in A_i\} = a_i$, and hence, by the Borel-Cantelli lemma,[2]

$$P\{x \in A_i \text{ i.o.}\} = 1 \tag{1}$$

if and only if

$$\sum_{i=1}^{\infty} a_i = \infty. \tag{2}$$

This can be stated, equivalently,

$$P\{x \notin A_i \quad \text{for all } i\} > 0 \tag{3}$$

if and only if

$$\sum_{i=1}^{\infty} a_i < \infty. \tag{4}$$

Applying Fubini's theorem, we obtain, from the preceding:

The divergence of the series $\sum a_i$ *is necessary and sufficient in order that the arcs* A_i *cover almost all*[3] *of* C *with probability* 1.

Thus equation (2) is necessary and sufficient in order that almost all of C be covered for almost all choices of centers (in C^∞ with appropriate measure and field

of measurable sets). An interesting question, which does not seem to have been considered previously, is whether the first "almost all" in the above assertion may be dropped. In other words, does equation (2) assure that the whole of C is covered with probability 1?

The negative answer to this query is perhaps the most interesting result of the present communication. If $\sum a_i$ (when rearranged as a series of decreasing terms) diverges very slowly, then there is a positive probability that C contains points not covered by any of the arcs A_i. On the other hand, if the divergence of $\sum a_i$ is not too slow, this does not occur.

Unfortunately, we do not know necessary and sufficient conditions on the rate of divergence of $\sum a_i$ which would insure that all of C is covered with probability 1. We shall therefore refrain in this first treatment of the subject from using heavier analysis and content ourselves with the two theorems below.

II

THEOREM 1. *Let the sequence* $(a_i)_{i=1}^{\infty}$ *be monotone nonincreasing and satisfy*

$$\overline{\lim_{i \to \infty}} \left(i a_i - 2 \log \frac{1}{a_i} \right) > -\infty ; \tag{5}$$

then

$$P\{\text{all of } C \text{ covered i.o.}\} = 1. \tag{6}$$

Thus, for example, relation (6) holds if $a_i \geq 2i^{-1} \log i$ for all large i.

Proof: If relation (5) holds, then there exists an increasing sequence of positive integers $(k_j)_{j=1}^{\infty}$ for which

$$(k_{j+1} - k_j) a_{k_{j+1}} - 2 \log \frac{1}{a_{k_{j+1}}} > -H > -\infty \tag{7}$$

for all j.

Consider now the arcs A_i with $k_j \leq i < k_{j+1}$. Divide the circumference C into[4]

$$n_j = \left[\frac{2}{a_{k_j+1}} \right] + 1 \tag{8}$$

equilong arcs, which we shall call "cells." (For definiteness, assume that all cells are semiopen on the same side.) If the center of an arc A_i with $i < k_{j+1}$ falls in a cell, then the arc covers all this cell. Hence, if E_j denotes the event that each of the above n_j cells contains the center of at least one of the arcs A_i with $k_j \leq i < k_{j+1}$, we have

$$P\left\{ C \subset \bigcup_{i=k_j}^{k_{j+1}-1} A_i \right\} \geq P\{E_j\}. \tag{9}$$

But it is well known[5] that if r objects are put into n cells, then the probability $p(r, n)$ that none of the cells is empty is given by

$$p(r, n) = \sum_{v=0}^{n} (-1)^v \binom{n}{v} \left(1 - \frac{v}{n}\right)^r. \tag{10}$$

Moreover, putting

$$\lambda = ne^{-r/n}, \tag{11}$$

we have

$$\lim p(r, n) = e^{-\lambda} \tag{12}$$

uniformly as $n \to \infty$.

We may, of course, assume that a_i tends to zero; then $n_j \to \infty$, and taking $r_j = k_{j+1} - k_j$, we have, from relation (7),

$$n_j e^{-r_j/n_j} < H' < \infty \tag{13}$$

for all j. Hence, by equations (11) and (12),

$$p\{E_j\} \geq p(r_j, n_j) > \delta > 0 \tag{14}$$

with suitable δ. Since the events E_j as well as those on the left-hand side of relation (9) are independent, Theorem 1 follows from relations (9) and (14) and the Borel-Cantelli lemma.

III

THEOREM 2. *There exist sequences* $(a_i)_{i=1}^{\infty}$ *satisfying equation* (2) *for which*

$$P\left\{C \subset \bigcup_{i=1}^{\infty} A_i\right\} < 1, \tag{15}$$

or, equivalently,

$$P\{\text{all of } C \text{ covered i.o.}\} = 0. \tag{16}$$

The following proof can easily be modified to yield an explicit slow divergence rate of $\sum a_i$ which would entail relations (15) and (16). But, as stated in Section I, we do not do this here.

Proof: From equations (11) and (12) it follows, in particular, that

$$\lim_{n \to \infty} p(n, [\epsilon n]) = 0 \tag{17}$$

for every $\epsilon > 0$. Let $(n_j)_{j=0}^{\infty}$ be a sequence of increasing integers, with $n_0 = 1$, such that n_j is divisible by $2n_{j-1}$ $(j = 1, 2, \ldots)$, and, moreover,

$$p\left(n_j, \frac{n_j}{2n_{j-1}}\right) < 2^{-j} \qquad (j = 1, 2, \ldots) \tag{18}$$

In view of equation (17) such a sequence does exist.

We claim that if B is any arc of C of length $1/n_{j-1}$ and if $B_1, \ldots, B_{n_j}$ are arcs of length $1/n_j$ whose centers are uniformly and independently distributed on C, then there is a probability $>1 - 2^{-j}$ that B contains a closed subarc B' of length $1/n_j$ which is disjoint from all B_i, i.e., such that

$$B' \cap B_i = \phi \qquad (i = 1, \ldots, n_j) \tag{19}$$

Indeed, divide B into $n_j/2n_{j-1}$ equilong arcs each of length $2/n_j$. Call the open arcs thus obtained "cells." If the centers of $B_1, \ldots, B_{n_j}$ were uniformly distributed

on B, then there would be, according to relation (18), a probability $>1 - 2^{-j}$ that at least one of these cells does not contain a center of any of the arcs. Since the centers are uniformly distributed over all C, this is a fortiori so. Since the length of the arcs B_i is $1/n_j$ and the cells are twice as long, it follows that if a cell contains no center of the arcs B_i, then an arc B' concentric to the cell and of length $1/n_j$ is disjoint from all B_i. This proves our assertion (since the probability that a center of any of the arcs coincides with a boundary point between two cells is zero, no harm was done in neglecting these points, and B' may be considered closed).

Now put

$$N_j = \sum_{\nu=0}^{j} n_\nu \qquad (j = 0, 1, 2, \ldots), \tag{20}$$

and let

$$a_i = \frac{1}{n_j} \qquad \text{for} \qquad N_{j-1} \leq i < N_j \quad (j = 1, 2, \ldots). \tag{21}$$

The series $\sum a_i$ thus constructed is clearly monotone and divergent. We maintain that if the a_i represent the lengths of the arcs A_i, then relations (15) and (16) hold.

Indeed, according to what has been shown above, there is a probability greater than $\alpha = \prod_{j=1}^{\infty} (1 - 2^{-j}) > 0$ that there exists a sequence B_j' $(j = 1, 2, \ldots)$ of closed arcs of length $1/n_j$ satisfying

$$B_1' \supset B_2' \supset \ldots \supset B_j' \supset \ldots \tag{22}$$

and having the property that

$$B_j' \cap \left(\bigcup_{i=1}^{N_j - 1} A_i \right) = \phi. \tag{23}$$

Hence, if $x = \bigcap_{j=1}^{\infty} B_j'$, we have $P\left\{x \notin \bigcup_{i=1}^{\infty} A_i\right\} > \alpha$. This proves relation (15), and equation (16) follows by the usual zero or one argument.

IV

It is not difficult to improve the above theorems and also to extend them to analogous problems where the centers are nonuniformly distributed, etc. Among the applications of the results proved here, we mention the following: Let b_i $(i = 1, 2, \ldots)$ be a sequence of complex numbers satisfying $|b_i| \geq |b_{i+1}|$ for all i. Then the divergence of $\sum |b_i|^2$ does not entail that almost all series $\sum \pm b_i z^i$ diverge everywhere on $|z| = 1$, but if $\sum |b_i|^\gamma$, with a suitable $\gamma > 2$, is divergent, then almost all series $\sum \pm b_i z^i$ are divergent everywhere on $|z| = 1$. (The signs $\pm$ are chosen independently; it is well known that $\sum |b_i|^2 = \infty$ entails, even without the assumption $|b_i| \geq |b_{i+1}|$, the divergence of almost all series $\sum \pm a_i z^i$ almost everywhere on $|z| = 1$.)

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[1] By $P\{\}$ is denoted the probability of the event within the braces.

[2] The abbreviation "i.o." indicates "infinitely often," that is, for infinitely many i. Similarly, in equations (6) and (16) it indicates that every point of C is covered by infinitely many A_i.

[3] In the Lebesgue sense.

[4] Square brackets, here and in equation (17), denote the integral part.

[5] R. von Mises, "Über Aufteilungs- und Besetzungswahrscheinlichkeiten, *Revue de la Faculté des Sciences de l'Université d'Istanbul*, new ser., **4**, 1–19, 1939. See also W. Feller, *Probability Theory and Its Applications*, New York, 1950, p. 72, where a different proof is given.

COMBINATORIAL PROBLEMS IN THE THEORY OF GRAPHS. II

BY G. W. FORD,* R. Z. NORMAN, AND G. E. UHLENBECK

INSTITUTE FOR ADVANCED STUDY, PRINCETON, NEW JERSEY, DEPARTMENT OF MATHEMATICS, PRINCETON UNIVERSITY, DEPARTMENT OF PHYSICS, UNIVERSITY OF MICHIGAN

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1. *Introduction: The Polya "Hauptsatz."*—In a previous note[1] we have given the solution to a number of counting problems for linear graphs with labeled points. The corresponding counting problems for graphs with indistinguishable points will be the subject of this note. For definitions and notation we refer to Paper I.

The main tool used in our discussion of the counting problem for unlabeled graphs is the *Hauptsatz* of Polya's paper.[2] Since it is so important, we shall give a brief statement of this theorem:

Consider first a collection of "figures," each figure being assigned an ordered pair of integers, called the "content" of the figure.[3] Let $f(n, m)$ be the number of figures in the collection with content (n, m), and define the figure counting series

$$f(x, y) = \sum_{n=0}^{\infty} \sum_{m=0}^{\infty} f(n, m) x^n y^m. \tag{1}$$

Next consider a set of p "points," along with a group G of permutations of these points. A "configuration" is obtained by hanging figures from the collection on the p points, repetitions allowed. Two configurations are said to be equivalent if there is a permutation of G taking one into the other, and the content of a configuration is the sum of the contents of its figures. Denoting by $F(n, m)$ the number of inequivalent configurations of content (n, m), the configuration counting series is defined as

$$F(x, y) = \sum_{n=0}^{\infty} \sum_{m=0}^{\infty} F(n, m) x^n y^m. \tag{2}$$

The Polya Hauptsatz states that the expression for $F(x, y)$ in terms of $f(x, y)$ and the group G is

$$F(x, y) = Z(G, f(x, y)), \tag{3}$$

where $Z(G, f(x, y))$ denotes the cycle index[4] of G in which the variable f_k is replaced by $f(x^k, y^k)$.

2. *The Number of Rooted Star Trees.*—As in Paper I, to simplify the writing, we will present the method for counting *pure* star trees. Let $\Theta(n)$ be the number of

rooted pure star trees with indistinguishable points which are composed of n stars joined together by articulation points. We introduce the counting series[5]

$$\Theta(y) = \sum_{m=0}^{\infty} \Theta(n) y^n. \tag{4}$$

Next we introduce $\Theta_m(y)$, the counting series for rooted star trees with exactly m main leaves. Clearly,

$$\Theta(y) = \sum_{m=0}^{\infty} \Theta_m(y), \tag{5}$$

with $\Theta_0(y) \equiv 1$. Now rooted star trees with m main leaves may be constructed by selecting m rooted star trees with *one* main leaf and hanging them all on the root. We may use the Polya Hauptsatz in which the figure collection is the collection of rooted star trees with one main leaf, the content is the number of stars in such a star tree, the "points" are the root considered m-fold, and the group G is the symmetric group S_m of degree m. The Polya theorem then gives the counting series for configurations which are rooted star trees with m main leaves:

$$\Theta_m(y) = Z(S_m, \Theta_1(y)). \tag{6}$$

We next observe that rooted star trees with *one* main leaf may be constructed by starting with the main leaf and hanging rooted star trees on the points other than the root. Thus $\Theta_1(y)$ can be expressed in terms of $\Theta(y)$ by using the Polya Hauptsatz in which the figure collection is the collection of all rooted star trees, the content is the number of stars in a rooted star tree, the "points" are the points of the main leaf other than the root, and the group G is the group obtained from the group Γ of the main leaf star by fixing one point, the root. We call this group a "derived group" of the main leaf.[6] If all the points of the main leaf are similar,[7] there is only one derived group; otherwise, there is one corresponding to each class of similar points. We denote the derived group corresponding to the qth class of similar points by $\Gamma^{(q)}$. Since a point from each class of similar points of the main leaf may in turn be taken as the root, the Hauptsatz gives

$$\Theta_1(y) = y \sum_q Z(\Gamma^{(q)}, \Theta(y)), \tag{7}$$

where the sum extends over the classes of similar points, and the factor y accounts for the main leaf.

We introduce the notation $Z'(\Gamma) = \sum_q Z(\Gamma^{(q)})$, which is justified by the identity[8]

$$Z'(\Gamma) = \frac{\partial}{\partial f_1} Z(\Gamma). \tag{8}$$

Using in addition the identity

$$\sum_{m=0}^{\infty} Z(S_m) = \exp \sum_{k=1}^{\infty} \frac{1}{k} f_k, \tag{9}$$

with $Z(S_0) = 1$, and combining equations (5), (6), and (7), we obtain the final result

$$\Theta(y) = \exp \sum_{k=1}^{\infty} \frac{y^k}{k} Z'(\Gamma, \Theta(y^k)). \tag{10}$$

The results previously obtained by Harary and Uhlenbeck[9] for rooted Husimi trees are a special case of equation (10).

The generalization to the case of mixed star trees is again straightforward. Let $\Theta(n_1, n_2, \ldots)$ be the number of rooted star trees with unlabeled points composed of n_1 stars of type 1, n_2 stars of type 2, etc. Introduce the counting series

$$\Theta(y_1, y_2, \ldots) = \underset{[n_\sigma]}{S} \Theta(n_1, n_2, \ldots) {y_1}^{n_1} {y_2}^{n_2} \ldots, \tag{11}$$

where the round sum goes over all sets $[n_\sigma]$. By an immediate extension of the reasoning given above for the pure star trees, one obtains the result corresponding to equation (10):

$$\Theta(y_1, y_2, \ldots) = \exp \sum_{k=1}^{\infty} \frac{1}{k} \sum_{\sigma} {y_\sigma}^k Z'(\Gamma_\sigma, \Theta({y_1}^k, {y_2}^k, \ldots)), \tag{12}$$

where Γ_σ is the group of a star of type σ. From this equation one can derive recursion relations for the numbers $\Theta(n_1, n_2, \ldots)$, but it is not possible to obtain an explicit expression for these numbers, as was the case in Paper I for the corresponding problem for graphs with labeled points.

The result (12) was first obtained by Norman and appears in the theses of Norman and Ford.[8]

3. *The Number of Free Star Trees.*—Again we will consider first the case of pure star trees. Let $\theta(n)$ be the number of pure free star trees containing n stars. We will derive an expression for the corresponding counting series $\theta(y)$ in terms of the counting series $\Theta(y)$ for rooted star trees; this expression is

$$\theta(y) = \Theta(y) - y[\Theta(y)\, Z'(\Gamma, \Theta(y)) - Z(\Gamma, \Theta(y))], \tag{13}$$

where Γ is the group of the star.

The first step in the proof of equation (13) is to establish a relation corresponding to equation (6) in Paper I. Let p be the number of dissimilar points of a star tree, and let s be the number of dissimilar stars in this tree;[10] then

$$1 = p - \sum_{k=1}^{s} (p_k - 1), \tag{14}$$

where p_k is the number of dissimilar points in the kth dissimilar star. To obtain this relation, one chooses an end star and deletes it and all stars similar to it, leaving their articulation points.[11] This removes $(p_1 - 1)$ dissimilar points from the star tree and leaves $(s - 1)$ dissimilar stars. This process is repeated until only one class of similar stars is left, which contains p_s dissimilar points. Hence the total numbers of dissimilar points p is given by

$$p = p_s + \sum_{k=1}^{s-1} (p_k - 1),$$

which is equivalent to equation (14).

If we sum equation (14) over all star trees with n stars, we obtain

$$\theta(n) = \Theta(n) - \left[\underset{(T_n)}{S} \left(\sum_{k=1}^{s} p_k \right) - \underset{(T_n)}{S}\, s \right], \tag{15}$$

where the round sum extends over all star trees with n stars. Denoting the first term in the brackets by $A(n)$ and the second by $B(n)$, we shall show how the Polya Hauptsatz can be used to determine these numbers.

$B(n)$ is the total number of dissimilar stars in all free star trees with n stars. Looked at another way, $B(n)$ is also the number of ways rooted star trees can be hung on the points of a designated star to obtain a star tree with n stars. Introducing the counting series $B(y)$, an application of the Polya theorem yields

$$B(y) = y\, Z(\Gamma, \Theta(y)), \tag{16}$$

where the factor y accounts for the designated star.

Finally, $A(n)$ is the total number of dissimilar points in all free star trees with n stars, each point counted once for *each* dissimilar star on which it lies. Looked at another way, $A(n)$ is the number of ways rooted star trees can be hung on the points of a designated star in which one point has been singled out. The counting series for rooted star trees which may be attached to the singled-out point is just $\Theta(y)$, while that for the configuration of rooted star trees attached to all other points of the designated star is the counting series for rooted star trees with one main leaf, which is given by equation (7). Using the product property, we get

$$A(y) = y\, \Theta(y)\, Z'(\Gamma, \Theta(y)). \tag{17}$$

Expressing relation (15) in terms of counting series and using equations (16) and (17), we obtain equation (13).

The generalization of equation (13) to the case of mixed star trees is straightforward. If $\theta(n_1, n_2, \ldots)$ is the number of free star trees containing n_1 stars of type 1, n_2 stars of type 2, etc., then for the corresponding counting series $\theta(y_1, y_2, \ldots)$ one has

$$\theta(y_1, y_2, \ldots) = \Theta(y_1, y_2, \ldots) - \sum_{\sigma} y_\sigma [\Theta(y_1, y_2, \ldots)\, Z'(\Gamma_\sigma, \Theta(y_1, y_2, \ldots) - Z(\Gamma_\sigma, \Theta(y_1, y_2, \ldots))]. \tag{18}$$

This relation was first obtained by Norman,[8] and the presentation here follows more closely that given by Ford.[8] A more complete discussion of the material in Sections 2 and 3 appears in a forthcoming paper.[12]

4. *The Numbers of Graphs and of Connected Graphs with p Unlabeled Points and k Lines.*—These numbers, which we will denote by $\pi(p, k)$ and $\gamma(p, k)$, were first obtained by Polya[13] and Riddell,[14] respectively. In this section we will present expressions for the counting series

$$\pi(x, y) = \sum_{p=1}^{\infty} \pi(p; y)x^p = \sum_{p=1}^{\infty} \sum_{k=0}^{p(p-1)/2} \pi(p, k)x^p y^k, \tag{19}$$

$$\gamma(x, y) = \sum_{p=1}^{\infty} \sum_{k=p-1}^{p(p-1)/2} \gamma(p, k)x^p y^k \tag{20}$$

giving only enough discussion to make the meaning clear. For a complete exposition we refer to the paper of Harary.[14]

We use the Polya Hauptsatz to obtain the configuration series $\pi(p; y)$. The figure collection consists of two figures, a pair of points joined by a line, and a pair of points not joined by a line. The content of a figure is the number of lines in it; thus the figure counting series is $1 + y$. The "points" of the Polya theorem are the $p(p-1)/2$ pairs of points of the graph. Two graphs of p points are equivalent if there is a permutation of the points carrying one into the other; therefore, the group of the Hauptsatz is the group of permutations of the $p(p-1)/2$ pairs of points induced by the symmetric group, S_p, of the points themselves. Denoting the cycle index of this *point pair group* by $Z_2(S_p)$, we obtain the result

$$\pi(p; y) = Z_2(S_p, 1 + y). \tag{21}$$

For an explicit expression for $Z_2(S_p)$ see Harary[14] and Ford.[8]

An expression relating $\pi(x, y)$ and $\gamma(x, y)$ may be obtained by a method entirely similar to that used in obtaining relation (18) of Paper I. The result is

$$\sum_{k=1}^{\infty} \frac{1}{k} \gamma(x^k, y^k) = \ln [1 + \pi(x, y)]. \tag{22}$$

Tables of $\pi(p, k)$ and $\gamma(p, k)$ are given through $p = 7$ in the dissertation of Riddell.[14] The numbers $\pi(p, k)$ are also given through $p = 7$ by Harary.[14]

The determination of the number, $\sigma(p, k)$, of stars with p unlabeled points and k lines is an open question.

* On leave of absence from the University of Notre Dame.

[1] G. W. Ford and G. E. Uhlenbeck, "Combinatorial Problems in the Theory of Graphs. I," these PROCEEDINGS, **42**, 122–128, 1956; hereafter referred to as "Paper I."

[2] G. Polya, "Kombinatorische Anzahlbestimmungen für Gruppen, Graphen, und chemische Verbindungen," *Acta Math.*, **68**, 145, 1937.

[3] The generalization to a content which is any ordered set of integers will be obvious.

[4] The cycle index of a permutation group G is defined as

$$Z(G) = \frac{1}{g} \mathcal{S}' g_{j_1, j_2, \ldots j_s} f_1^{j_1} f_2^{j_2} \ldots f_s^{j_s}$$

where g is the order and s the degree of G; $f_1, f_2, \ldots, f_s$ are s variables, and $g_{j_1, j_2, \ldots j_s}$ is the number of elements of G which consist of j_1 cycles of length 1, j_2 cycles of length 2, etc. The round sum is over all sets $[j_k]$ consistent with the condition (denoted by the prime) $1j_1 + 2j_2 + \ldots + sj_s = s$. For example, if G is the symmetric group of degree s,

$$g_{j_1 j_2, \ldots j_s} = \frac{s!}{1^{j_1} j_1!\, 2^{j_2} j_2! \ldots s^{j_s} j_s!}.$$

[5] The counting series for graphs with unlabeled points defined in this manner satisfy the product property without the $1/p!$ introduced in the corresponding counting series for graphs with labeled points in Paper I. In Paper I, because the $1/p!$ was necessary, we were led to count according to the number of points of the graph. Here it is convenient to define the counting series in terms of the number of stars in the star tree; the two modes of counting are obviously equivalent.

[6] Considered as an abstract group, such a derived group is a subgroup of Γ. It is convenient to consider a derived group as operating on the points other than the root; hence, as a permutation group, it is of degree one less than Γ.

[7] In general, two points of a graph are similar if an operation of the group of the graph carries one into these. Here we are considering similarity with regard only to the main leaf rather than with regard to the star tree as a whole.

[8] G. W. Ford, "On the Theory of Linear Graphs, with Applications to the Theory of Condensation" (dissertation University of Michigan, 1954); R. Z. Norman, "On the Number of Linear Graphs with Given Blocks" (dissertation, University of Michigan, 1954).

[9] F. Harary and G. E. Uhlenbeck, "On the Number of Husimi Trees. I," these PROCEEDINGS, **39**, 315, 1953.

[10] Two stars are similar if an operation of the group of the star tree carries one star into the other.

[11] In this process the original similarity classes are to be preserved.

[12] R. Z. Norman "On the Number of Graphs with Given Blocks" (submitted to *Trans. Am. Math. Soc.*).

[13] G. Polya, unpublished.

[14] R. J. Riddell, "Contributions to the Theory of Condensations" (dissertation, University of Michigan, 1951); see also F. Harary, "The Number of Linear, Directed, Rooted, and Connected Graphs," *Trans. Am. Math. Soc.*, **78**, 445, 1955.

ON THE LITTLEWOOD-PALEY FUNCTION $g^*(\theta)$

BY A. ZYGMUND*

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1. Let H^λ, $\lambda > 0$, denote the class of functions

$$\Phi(z) = \sum_0^\infty c_n z^n$$

regular in $|z| < 1$ and such that

$$\mathfrak{M}_\lambda[\Phi(re^{i\theta})] = \{(2\pi)^{-1} \int_0^{2\pi} |\Phi(re^{i\theta})|^\lambda \, d\theta\}^{1/\lambda} < M < \infty$$

for some M independent of r. The partial sums of the series $\sum c_n e^{in\theta}$ we denote by $s_n(\theta)$, and the $(C, 1)$ means by $\sigma_n(\theta)$, and write $\Phi(e^{\theta}) = \lim_{r \to 1} \Phi(re^{i\theta})$.

In their work[1] on Fourier series Littlewood and Paley introduced the functions

$$g(\theta) = g(\theta, \Phi) = \{\int_0^1 (1 - \rho) |\Phi'(\rho e^{i\theta})|^2 \, d\rho\}^{1/2}, \tag{1.1}$$

$$g^*(\theta) = g^*(\theta, \Phi) = \{\int_0^1 (1 - \rho)\chi^2(\rho, \theta) d\rho\}^{1/2}, \tag{1.2}$$

where

$$\chi(\rho, \theta) = \{\pi^{-1} \int_0^1 |\Phi'(\rho e^{i(\theta + t)})|^2 \, P(\rho, t) dt\}^{1/2}$$

and $P(\rho, t) = {}^1/_2 (1 - \rho^2)/(1 - 2\rho \cos t + \rho^2)$. They showed that

$$\mathfrak{M}_\lambda[g] \leq A_\lambda \, \mathfrak{M}_\lambda[\Phi(e^{i\theta})] \tag{1.3}$$

for all $\lambda > 0$ and that

$$\mathfrak{M}_\lambda[g^*] \leq A_\lambda \, \mathfrak{M}_\lambda[\Phi(e^{i\theta})] \tag{1.4}$$

for $\lambda = 2, 4, \ldots$, and raised the problem of the validity of relation (1.4) for other values of λ.

That relation (1.4) holds for all $\lambda > 1$ was shown in an earlier paper[2] by a rather laborious argument, which, moreover, left the case $\lambda = 1$ open (the example $\Phi(z) = 1/(1 - z)$ shows that the inequality fails for $\lambda < 1$; we omit the simple computa-

tion). It turns out, however, that the problem is easily reducible to one solved a long time ago, and this approach gives a more complete answer, namely, the following:

THEOREM 1. *We have*

$$\mathfrak{M}_\lambda[g^*] \leq A_\lambda \mathfrak{M}_\lambda[\Phi(e^{i\theta})], \lambda > 1, \tag{1.5}$$

$$\mathfrak{M}[g^*] \leq A \int_0^{2\pi} |\Phi(e^{i\theta})| \log^+ |\Phi(e^{i\theta})| \, d\theta + A, \tag{1.6}$$

$$\mathfrak{M}_\mu[g^*] \leq A_\mu \mathfrak{M}[\Phi(e^{i\theta})], 0 < \mu < 1, \tag{1.7}$$

where $\mathfrak{M}$ *stands for* $\mathfrak{M}_1$.

Inequalities (1.6) and (1.7) seem to be new. The proof of Theorem 1 is based on the following simple observation that $g^*(\theta)$ is essentially identical with the function

$$\gamma(\theta) = \gamma(\theta, \Phi) = \left(\sum_0^\infty \frac{|s_n - \sigma_n|^2}{n+1} \right)^{1/2}, \tag{1.8}$$

which was known to satisfy inequalities analogous to relations (1.5), (1.6), and (1.7) (see an earlier paper[3] [Part I]). For first we observe that

$$g^*(\theta) = \left\{ \pi^{-1} \frac{1}{2} \int_0^1 (1+\rho)(1-\rho)^2 \, d\rho \int_0^{2\pi} \frac{|\Phi'(\rho e^{i(\theta+\psi)})|^2}{|1 - \rho e^{i\psi}|^2} \, d\psi \right\}^{1/2}. \tag{1.9}$$

Next, with $z = \rho e^{i\psi}$,

$$\begin{aligned} \sum_0^\infty s_n'(\theta) z^n &= \frac{d}{d\theta} \sum s_n(\theta) z^n = \frac{d}{d\theta} \{(1-z)^{-1} \sum c_n e^{in\theta} z^n\} \\ &= \rho i (1 - \rho e^{i\psi})^{-1} \Phi'(\rho e^{i(\theta+\psi)}) e^{i(\theta+\psi)} \end{aligned} \tag{1.10}$$

so that

$$\sum_1^\infty |s_n'(\theta)|^2 \rho^{2n} = (2\pi)^{-1} \rho^2 \int_0^{2\pi} |1 - \rho e^{i\psi}|^{-2} |\Phi'(\rho e^{i(\theta+\psi)})|^2 d\psi.$$

If we multiply this by $\rho^{-2}(1-\rho)^2$ and integrate over $0 < \rho < 1$, we get

$$\sum_1^\infty \frac{|s_n'(\theta)|^2}{(2n-1)2n(2n+1)} = (2\pi)^{-1} \int_0^1 (1-\rho)^2 \, d\rho \int_{-\pi}^{\pi} \frac{|\Phi'(\rho e^{i(\theta+\psi)})|^2}{|1 - \rho e^{i\psi}|^2} \, d\psi. \tag{1.11}$$

On the other hand,

$$\sum_1^\infty \frac{|s_n(\theta) - \sigma_n(\theta)|^2}{n+1} = \sum_1^\infty \frac{|s_n'(\theta)|^2}{(n+1)^3}. \tag{1.12}$$

If we compare this with the left side of equation (1.11) and compare equation (1.9) with the right side of equation (1.11), we notice that $g^*(\theta)/\gamma(\theta)$ is contained between two positive absolute constants, and if we use inequalities (1.5), (1.6), and (1.7) for γ,[4] Theorem 1 follows.

2. We now consider the function

$$g_\sigma^*(\theta) = \{ \int_0^1 \delta^\sigma \, d\rho \int_{-\pi}^{\pi} |1 - \rho e^{i\psi}|^{-\sigma} |\Phi'(\rho e^{i(\theta+\psi)})|^2 d\psi \}^{1/2} \tag{2.1}$$

(introduced in an earlier paper[2]), where $\delta = 1 - \rho$. If $\sigma = 2$, this function reduces

(except for the harmless factor $\frac{1}{2}\pi^{-1}(1+\rho)$) to $g^*(\theta)$, and from now on we use $g_2^*(\theta)$ for $g^*(\theta)$.

We shall denote by $H^\lambda \log^+ H$ the class of functions $\Phi(z) = \sum c_n z^n$, such that the integral

$$\int_0^{2\pi} |\Phi(re^{i\theta})|^\lambda \log^+ |\Phi(re^{i\theta})|\, d\theta$$

remains bounded for $r \to 1$. The following theorem generalizes Theorem 1.

THEOREM 2. *If $\Phi \in H^\lambda$, $0 < \lambda \leq 1$, then $g_{2/\lambda}^*$ is finite almost everywhere, and*

$$\mathfrak{M}_{\eta\lambda}[g_{2/\lambda}^*] \leq A_\eta\, \mathfrak{M}_\lambda[\Phi(e^{i\theta})], \qquad 0 < \eta < 1. \tag{2.2}$$

If $\Phi \in H^\lambda \log^+ H$, then

$$\mathfrak{M}_\lambda^\lambda[g_{2/\lambda}^*] \leq A_\lambda \int_0^{2\pi} |\Phi(e^{i\theta})|^\lambda \log^+ |\Phi(e^{i\theta})|\, d\theta. \tag{2.3}$$

The theorem holds for $\lambda = 1$ (see Theorem 1), and we deduce from this the case $\lambda < 1$ by a familiar argument (the argument which follows is taken from an earlier paper,[2] where it was applied to a function G_σ which is the dominant part of g_σ^*).

Suppose first that Φ has no zeros, so that $\Psi = \Phi^\lambda$ is in H. Integral (2.1), with $\sigma = 2/\lambda$, becomes

$$\left(\frac{2}{\lambda}\right)^2 \int_0^1 \delta^{2/\lambda}\, d\rho \int_{-\pi}^{\pi} |1 - \rho e^{i\chi}|^{-2/\lambda} |\Psi(\rho e^{i(\theta+\psi)})|^{2(-1+1/\lambda)} |\Psi'|^2\, d\psi \tag{2.4}$$

Write $\xi(\theta) = \sup |h^{-1} \int_\theta^{\theta+h} |\Psi(e^{it})|\, dt|$ for all $h \neq 0$. It is well known[5] that

$$|\Psi(\rho e^{i(\theta+\psi)})| \leq A\, \xi(\theta)\, \delta^{-1} |1 - \rho e^{i\psi}|, \tag{2.5}$$

so that integral (2.4) is majorized by

$$A_\lambda \{\xi(\theta)\}^{2(-1+1/\lambda)} \int_0^1 \delta^2\, d\rho \int_{-\pi}^{\pi} |1 - \rho e^{i\psi}|^{-2} |\Psi'(\rho e^{i(\theta+\psi)})|^2\, d\chi,$$

and we obtain the inequality

$$g_{2/\lambda}^*(\theta, \Phi) \leq A_\lambda \{\xi(\theta)\}^{(1-\lambda)/\lambda} g_2^*(\theta, \Psi). \tag{2.6}$$

Since, by Theorem 1, $g_2^*(\theta, \Psi)$ is finite almost everywhere, the same holds for $g_{2/\lambda}^*(\theta, \Phi)$. Observe now that by the Hardy-Littlewood maximal theorem

$$\mathfrak{M}_\eta[\xi] \leq A_\eta\, \mathfrak{M}[\Psi(e^{i\theta})], \qquad \mathfrak{M}[\xi] \leq A \int_0^1 |\Psi(e^{i\theta})| \log^+ |\Psi|\, d\theta + A. \tag{2.7}$$

Raising relation (2.6) to the power $\eta\lambda$, applying Hölder's inequality with exponents $1/(1-\lambda)$ and $1/\lambda$, and also the first inequality (2.7) (for Ψ), we immediately obtain relation (2.2). To obtain relation (2.3), we use the second inequality (2.7) and inequality (1.6) (for Ψ). Then, writing Φ, Ψ for $\Phi(e^{i\theta})$, $\Psi(e^{i\theta})$, we have

$$\begin{aligned}
\int_0^{2\pi} g_{2/\lambda}{}^{*\lambda}[\Phi]\, d\theta &\leq A_\lambda \int_0^{2\pi} \xi^{1-\lambda} g_2{}^{*\lambda}[\Psi]\, d\theta \leq A_\lambda \left(\int_0^{2\pi} \xi\, d\theta\right)^{1-\lambda} \left(\int_0^{2\pi} g_2{}^*[\Psi]\, d\ell\right)^\lambda \\
&\leq A_\lambda \left\{\int_0^{2\pi} |\Psi| \log^+ |\Psi|\, d\theta + A\right\}^{1-\lambda} \left\{A \int_0^{2\pi} |\Psi| \log^+ |\Psi|\, d\theta + A\right\}^\lambda \\
&= A_\lambda \int_0^{2\pi} |\Psi| \log^+ |\Psi|\, d\theta + A_\lambda \leq A_\lambda \int_0^{2\pi} |\Phi|^\lambda \log^+ |\Phi|\, d\theta + A_\lambda
\end{aligned}$$

This completes the proof of Theorem 2 in the case when Φ has no zeros. In the general case we have, as is well known, $\Phi = \Phi_1 + \Phi_2$, where Φ_1 and Φ_2 have no zeros and satisfy the inequalities $|\Phi_1| \leq 2|\Phi|$, $|\Phi_2| \leq 2|\Phi|$ in $|z| < 1$. Since, by

Minkowski's inequality, $g_\sigma^*(\Phi_1 + \Phi_2) \leq g_\sigma^*(\Phi_1) + g_\sigma^*(\Phi_2)$, the general result is an immediate consequence of the one just proved.

I do not know whether Theorem 2 holds for $1 < \lambda < 2$. For $\lambda = 2$ (and a fortiori for $\lambda > 2$) it fails, since there are functions Φ regular in $|z| < 1$, continuous in $|z| \leq 1$ and such that $g_1^*(\theta, \Phi) = \infty$ for all θ. It can be shown that if $1 < \lambda < 2$ and $\sigma > 2/\lambda$, then the function $g_\sigma^*(\theta, \Phi)$ is finite almost everywhere and satisfies $\mathfrak{M}_\lambda[g_\sigma^*] \leq A_{\lambda\sigma}\, \mathfrak{M}_\lambda[\Phi]$. The proof is given in an earlier paper[2] (pp. 178–179) for the function G_σ^* mentioned above, but the result has no interesting application.

3. Let $s_n^\alpha = s_n^\alpha(\theta, \Phi)$ and $\sigma_n^\alpha = \sigma_n^\alpha(\theta, \Phi)$ be the Cesàro sums and Cesàro means for the series $\sum c_n z^n$, $z = e^{i\theta}$. Hence

$$\sigma_n^\alpha(\theta) = \frac{s_n^\alpha(\theta)}{A_n^\alpha} = \frac{1}{A_n^\alpha} \sum_{n=0}^{n} A_{n-\nu}^\alpha\, c_\nu e^{i\nu\theta},$$

where $A_n^\alpha = (\alpha + 1)(\alpha + 2) \ldots (\alpha + n)/n!$. A simple computation shows that

$$\sigma_n^\alpha - \sigma_n^{\alpha+1} = \frac{1}{A_n^\alpha} (\alpha + n + 1)^{-1} \sum_0^n A_{n-\nu}^\alpha\, \nu c_\nu e^{i\nu\theta} = -\frac{i}{A_n^\alpha} (\alpha + n + 1)^{-1} (s_n^\alpha(\theta))', \tag{3.1}$$

so that

$$\sum_0^\infty (n + 1)^{-1} \left| \sigma_n^\alpha - \sigma_n^{\alpha+1} \right|^2 \leq C_\alpha \sum_0^\infty (n + 1)^{-2\alpha-3} \left| s_n^{\alpha\prime}(\theta) \right|^2. \tag{3.2}$$

Observe that, if $z = \rho e^{i\psi}$, then

$$\sum_0^\infty s_n^\alpha(\theta) z^n = (1 - z)^{-\alpha-1} \sum_0^\infty c_n e^{in\theta} z^n = (1 - \rho e^{i\psi})^{-\alpha-1}\, \Phi(\rho e^{i(\theta+\psi)}).$$

Hence, differentiating and using Parseval's formula,

$$\sum_1^\infty \left| s_n^{\alpha\prime}(\theta) \right|^2 \rho^{2n} = (2\pi)^{-1} \rho^{-2} \int_0^{2\pi} \left| 1 - \rho e^{i\psi} \right|^{-2\alpha-2} \left| \Phi'(\rho e^{i(\theta+\psi)}) \right|^2 d\theta. \tag{3.3}$$

If we multiply this by $\rho^{-2}(1 - \rho)^{2\alpha+2}$ and integrate over $0 \leq \rho < 1$, we get

$$\sum (n + 1)^{-2\alpha-3} \left| s_n^{\alpha\prime}(\theta) \right|^2 \leq C_\alpha\, g_{2\alpha+2}^{*2}(\theta, \Phi),$$

which, after relation (3.2), gives

$$\sum (n + 1)^{-1} \left| \sigma_n^\alpha(\theta) - \sigma_n^{\alpha+1}(\theta) \right|^2 \leq C_\alpha g_{2\alpha+2}^{*2}(\theta, \Phi). \tag{3.4}$$

Denote the square root of the left side by $\gamma_\alpha(\theta) = \gamma_\alpha(\theta, \Phi)$; thus γ_0 is our former function γ. Put $\alpha = (1/\lambda) - 1$; we therefore have

$$\gamma_\alpha(\theta) \leq C_\alpha g_{2/\lambda}^*(\theta, \Phi),$$

and we obtain in view of Theorem 2 the inequalities

$$\mathfrak{M}_{\lambda\eta}[\gamma_\alpha] \leq C_{\lambda,\eta}\, \mathfrak{M}_\lambda[\Phi],\ \alpha = \frac{1}{\lambda} - 1;\ 0 < \lambda \leq 1, \tag{3.5}$$

$$\mathfrak{M}_\lambda[\gamma_\alpha] \leq C_\lambda \left\{ \int_0^{2\pi} \left| \Phi(e^{i\theta}) \right|^\lambda \log^+ \left| \Phi \right| d\theta + 1 \right\}^{1/\lambda}. \tag{3.6}$$

In an earlier paper[3] (Part II) I proved that (*a*) if $\Phi = \sum c_n z^n$ is in H^λ, $0 < \lambda < 1$, then $\sum c_n e^{in\theta}$ is summable $(C, \alpha) = (C, (1/\lambda) - 1)$ almost everywhere. I also conjectured that (*b*) if $\Phi \in H^\lambda \log^+ H$, then $\sigma_*^\alpha(\theta) = \sup_r |\sigma_r^\alpha(\theta)|$ is in L^λ (I could prove the conjecture for $\lambda = 1/2, 1/3, 1/4, \ldots$ only, in which cases it is a relatively simple consequence of the result for $\lambda = 1$, established in Part I of an earlier paper[4]). Inequalities (3.5) and (3.6) contain the proofs of (*a*) and (*b*) for $0 < \lambda \leq 1/2$, but since we do not know whether Theorem 2 holds for $1 < \lambda < 2$, proposition (*b*) for $1/2 < \lambda < 1$ is still a conjecture.[6] I briefly indicate how inequality (3.5) implies (*b*) for $0 < \lambda \leq 1/2$.

We may suppose that Φ has no zeros, so that $\Phi = \Psi^2$, where $\Psi \in H^{2\lambda} \log^+ H$. Then

$$\sigma_n^\alpha[\Phi] = A_n^{-\alpha} s_n^\alpha[\Phi] = A_n^{-\alpha} \sum_{\nu=0}^{n} s_\nu^{1/2(\alpha-1)}[\Psi]\, s_{n-\nu}^{1/2(\alpha-1)}[\Psi],$$

$$|\sigma_n^\alpha[\Phi]| \leq (A_n^\alpha)^{-1} \sum_0^n |s_\nu^{1/2(\alpha-1)}[\Psi]|^2 \leq (A_n^\alpha)^{-1} \sum_0^n |\sigma_\nu^{1/2(\alpha-1)}[\Psi]|^2 (\nu+1)^{\alpha-1}$$

$$\leq (A_n^\alpha)^{-1} \sum_{\nu=0}^{n} |\sigma_\nu^{1/2(\alpha-1)}[\Psi] - \sigma_\nu^{1/2(\alpha+1)}[\Psi]|^2 (\nu+1)^{\alpha-1} + (A_n^\alpha)^{-1} \sum_0^n |\sigma_\nu^{1/2(\alpha+1)}[\Psi]|^2 (\nu+1)^{\alpha-1}.$$

Denote the upper bounds for $n = 0, 1, 2, \ldots$ of the last two terms by P and Q, respectively. Clearly,

$$P \leq C_\alpha \sum_1^\infty |\sigma_\nu^{1/2(\alpha-1)}[\Psi] - \sigma_\nu^{1/2(\alpha+1)}[\Psi]|^2 (\nu+1)^{-1} = C_\alpha \gamma_{1/2(\alpha-1)}{}^2[\Psi]$$

$$\leq C_\alpha \gamma_{\alpha+1}{}^{*2}[\Psi]$$

by relation (3.4), so that, by relation (2.3) applied to Ψ,

$$\mathfrak{M}_\lambda[P] \leq C_\alpha \mathfrak{M}_{2\lambda}{}^2[g_{\alpha+1}{}^*[\Psi]] = C_\alpha \mathfrak{M}_{2\lambda}{}^2[g_{1/\lambda}{}^*[\Psi]]$$
$$\leq C_\alpha [\int_0^{2\pi} |\Psi|^{2\lambda} \log^+ |\Psi|\, d\theta + 1]^{1/\lambda} \leq C_\alpha [\int_0^{2\pi} |\Phi|^\lambda \log^+ |\Phi|\, d\theta + 1]^{1/\lambda}. \tag{3.7}$$

On the other hand, clearly, $Q \leq \sigma_*^{1/2(\alpha+1)}[\theta, \Psi]$. Since the index $1/2(\alpha+1)$ of summability is higher than the critical index $1/2(\alpha-1)$, it is well known that $\mathfrak{M}_\lambda[Q] \leq C_\lambda \mathfrak{M}_{2\lambda}{}^2[\Psi] = C_\lambda \mathfrak{M}_\lambda[\Phi]$, which, combined with relation (3.7), shows that $\mathfrak{M}_\lambda[\sigma_*^\alpha[\Phi]]$ is majorized by the right side of relation (3.6).

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[4] See Part I, note 3.

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[6] Inequalities (3.5) and (3.6) can also be found in a recent paper of G. Sunouchi. My work was independent of his and was motivated by the desire to prove conjecture (*b*). I am presenting my partial results only because of the appearance of his work. ("On the Summability of Power Series and Fourier Series," *Tohoku Math. J.*, **7**, 96–109, 1955.)

*THE SENSITIVITY OF THE TURTLE'S EAR AS SHOWN BY ITS ELECTRICAL POTENTIALS**

By Ernest Glen Wever and Jack A. Vernon

PRINCETON UNIVERSITY

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Little is known about the performance of the ear of the turtle. Andrews[1] in 1915 succeeded in training five individuals of the genus *Chrysemys* to distinguish the sound of a whistle from that of a bell when one of these was made the positive signal for feeding. Poliakov[2] reported the establishment of conditioned head withdrawal in three specimens of *Emys orbicularis* in response to a variety of tones and noises. On the other hand, Kuroda[3] in two experiments, one of which used the same method as Andrews, failed to obtain any evidence of hearing in the Japanese turtle, *Clemmys japonica*.

Three experiments have dealt with the turtle's ear by electrophysiological methods. Foà and Peroni[4] in 1930 recorded from the facial-auditory nerve of the giant sea tortoise, *Thalassochelys caretta*, and obtained impulses on stimulation with strong tones from organ pipes with frequencies of $16^1/_2$, 33, and 132 cycles. Wever and Bray[5] in 1931 obtained synchronized responses from the eighth nerve of the painted turtle, *Chrysemys picta*, for all tones up to 1,000 or 1,200 cycles, though the responses were feeble beyond 500 cycles. Adrian, Craik, and Sturdy[6] obtained similar responses from the eighth nerve of the box turtle, *Cistudo*, and a land turtle *Testudo graeca*, on stimulation with tones up to 400 cycles, and in two specimens they obtained inner-ear potentials also for tones up to 700 or 800 cycles.

The electrophysiological experiments are in agreement in indicating that the turtle's ear is responsive to low tones. But despite this evidence and the positive results obtained by Andrews and Poliakov, many herpetologists have held the opinion that turtles are completely deaf and that their ear is only a vestigial organ. Such a view was expressed by Pope[7] in 1939 and by Carr[8] in 1952.

The present experiments have made use of the electrophysiological method, with measurements of the inner-ear potentials produced by sounds of known intensities. The results reported here were obtained on twenty-one animals belonging to three related species: four wood turtles, *Clemmys insculpta* (Le Conte), nine painted turtles, *Chrysemys picta picta* (Schneider), and eight "Cumberland" turtles, *Pseudemys scripta*. The individuals comprising the last group showed some race variations and included *P. scripta scripta* (Schoepff), *P. scripta elegans* (Wied), and *P. scripta troostii* (Holbrook), as well as some intergrades among these but for present purposes these variations are of no significance. All these species belong to the family Emydidae.

METHOD AND PROCEDURE

The animals were anesthetized with 25 per cent ethyl carbamate in turtle Ringer's solution, injected into the leg muscles in the amount of 6.5 cc. per kilogram body weight. The head was held by a clamp fixed to the lower jaw, and movement was further restricted by anchoring the shell and closing all leg openings with tape.

Three different positions for the active electrode were used, and the operative procedure varied accordingly. In these procedures bleeding was sometimes a

problem, and it was found helpful to tie a string tightly around the neck and to leave it in place for a short time—2–5 minutes, usually—until the bleeding stopped. This constriction did not entirely block the circulation to the head, and the animal remained in good condition. In a test experiment we found that leaving the ligature in place for as long as 90 minutes had no effect upon the inner-ear potentials. A radiofrequency cautery was useful also in controlling hemorrhage from small vessels.

Early in the experiment the active electrode was placed on the cranial wall of the otic capsule, near the point of penetration of the posterior root of the eighth nerve. Sometimes also it was placed in a small hole drilled in this wall. This approach requires a dorsal skin incision and removal of the bony cranial roof in the region of the cerebellum. It was found best to avoid any opening of the dura mater, which causes troublesome bleeding. The cerebellum can be pushed to one side, and then the two roots of the eighth nerve can be located deep in the cavity as they leave the otic capsule and enter the medulla. The best position for the indifferent electrode was found to be beneath the skin in the dorsal part of the cranium, at a point remote from any muscles.

Later on, a simpler and more satisfactory procedure was worked out for approaching the otic capsule. A skin incision was made along the dorsal crest of the squamous bone, at the most lateral insertion of the temporalis muscle, and then this muscle was elevated to expose the suture between the paroccipital and supraoccipital bones. Midway along this suture and extending medially is a clear area in the otherwise white bone, and this area marks the most dorsal course of the bony channel for the posterior semicircular canal. A fine steel needle, insulated except at the tip, was pushed into the suture at this place to make contact with the perilymph of the bony canal. The utricle also is close to the surface here, and the saccule, lagena, and cochlea are only about 3 mm. below, so that it is possible to record from such of these endings as are responsive to sounds. This dorsal location in the otic capsule was used in most of the experiments.

A third and more elaborate procedure, used only in a few ears, permitted a recording from the round window membrane. The posterior extension of the tympanic cavity (the so-called "mastoid antrum") is first entered by removing its dorsal wall, which is formed by the squamous bone. Then along the medial wall of this cavity there can be seen through the bone a broad dark line that represents the course of the cavernous sinus, in which are included the carotid artery, internal jugular vein, and facial nerve. By drilling into the bone just above this sinus, carefully avoiding its vessels, the pericapsular recess is entered. This recess is a bent tube that borders on the otic capsule along its posterior and lateral aspects and incloses both oval and round windows. It is filled with fluid. If the fluid is removed by applying pledgets of absorbent cotton, the round window membrane may be seen at the posterior end of the recess. This window bounds the fluid of the inner ear, just as in the higher forms. A platinum-foil electrode was passed through the exposed cavity of the recess to make contact with this membrane.

After the electrode was fixed in one of these positions, a sound tube leading from a loud-speaker was placed firmly against the head in such a manner as to inclose the tympanic membrane without touching it, with a piece of rubber tubing over the end to serve as a gasket. Further, to insure that the fit to the head was sound-

tight, the end of the rubber tube was covered with petroleum jelly, and sometimes strands of cotton covered with the jelly were wound around the end of the tube. A probe tube from a condenser microphone entered the sound tube from the side and then ran concentrically with this tube to a point near its tip, and by means of this probe tube and microphone the sound pressures applied to the tympanic membrane were measured.

As will be brought out presently, the inner-ear potentials of the turtle are small in magnitude, and, as usual in low-level recording, it is necessary to use precautions to avoid artifacts. The stimulating sounds were confined in tubes as described and were not permitted to pervade the experimental room. The condenser microphone and its associated apparatus were attached only for a calibration run at the beginning or the end of an experiment and were not in position near the ear during the measurements of its potentials. In spite of these precautions it was possible to obtain potentials representing the microphonic action of tissues when sounds of extreme intensities were applied, but these intensities were beyond any required for our measurements and indeed were such as to produce severe injury to the ear. With the sound intensities normally employed, these spurious potentials were not measurable, and numerous checks were applied from time to time throughout the experiment to guard against their appearance.

The stimulating tones usually extended from 100 to 3,000 cycles. Sometimes higher tones were used, but it was found that if such tones were delivered in sufficient strength to produce measurable potentials, they almost always caused injury to the ear, as shown by a reduction in the response to all tones. Our standard procedure for determining the sensitivity was to present various tones at the intensities necessary to produce a certain small potential, usually 0.3 or 0.1 microvolt, beginning with the low tones and then progressing upward toward the more hazardous frequencies. Often the high-tone presentations were alternated with retests at 400 cycles to discover whether any damage had been done. The results that are given for tones above 3,000 cycles were obtained at the end of the series of measurements on a given ear, so that other portions of the function would not be affected, with the presentations made as brief as possible to minimize the injury.

The measurements were made at a room temperature of about 20°–22° C.

RESULTS

General Physiological Conditions.—We shall begin with a consideration of the condition of the animal in relation to the ear's generation of potentials. It is common in experimental studies on reptiles to make no attempt to maintain the circulation, or otherwise to give attention to the general physiological condition. For example, in the experiments of Adrian, Craik, and Sturdy, mentioned above, the animal was decapitated, the head dissected widely, and the brain almost wholly removed. Nevertheless, these authors reported that the preparation remained in satisfactory condition for hours, and nothing was gained by attempts to preserve the circulation. It should be noted that in these experiments the recording was from the eighth nerve during stimulation with sounds that may have been of extreme intensities, and under such conditions a variation in the performance of the sensory cells may not have been revealed.

Our observations of the inner-ear potentials show an essential dependence upon the state of the animal. Two of our animals died in the course of the observations, evidently from an overdose of anesthetic, and in both of these the responses declined rapidly. In another animal, after the usual measurements had been made, a tone of 400 cycles was presented and steadily maintained at the intensity necessary to produce a response of 1 microvolt. Normally this response will continue for hours. In this instance, after a number of observations had been made to establish the stability of the response, the head was cut off and the observations continued over a period of 30 minutes. The results are given in Figure 1. As will be seen, the response failed rapidly and at the end of the 30-minute period had sustained a loss of 10 db. Moreover, at that time the decline was still continuing at a rapid rate. It is clear that for precise determinations of the performance of the turtle's ear it is necessary that the circulation be maintained and, in general, that the animal be kept in good condition.

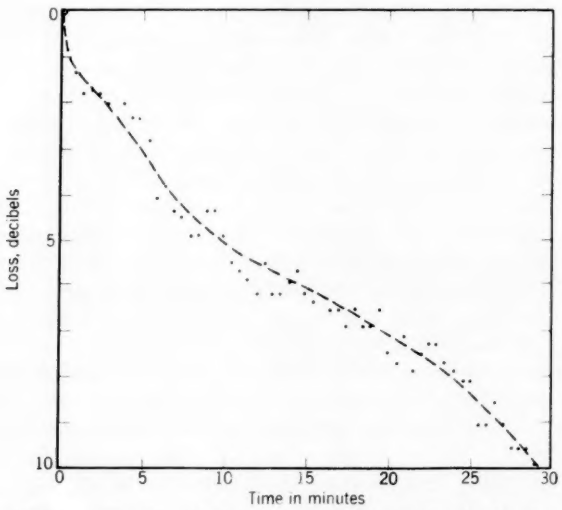

FIG. 1.—The loss of inner-ear potentials in the turtle after death. The test tone was 400 cycles, presented at a constant intensity.

Intensity Functions.—As has been found for mammals and birds, the inner-ear potentials show a progressive and regular increase with sound pressure at the lower levels and then at higher levels begin to bend, attain a maximum, and finally decline. Figure 2 shows some representative intensity functions. These curves are approximately linear in their lower portions: an increase of sound pressure by some fractional amount gives an increase of potential of nearly the same fractional amount. In these curves the departure from linearity in the upper portion of the curve is rather gradual. This is the typical condition, though for some of the functions, more often for the high frequencies, the decline beyond the maximum is more rapid.

Sensitivity Functions.—The next three figures present sensitivity curves for representative ears among the three species studied. Each figure contains three curves for different animals. Two of these (*solid and short-dashed lines*) were obtained with the active electrode in the dorsal position in the otic capsule, and the remaining one (*long-dashed line*) with the electrode on the cranial wall. The solid line represents one of the most sensitive animals of its group, and the short-dashed line a more typical animal. Figure 3 shows results for *Chrysemys picta*, Figure 4 for *P. scripta*, and Figure 5 for *Clemmys insculpta*. These curves show the sound pressures required at various frequencies to produce a constant potential of 0.3 microvolt.

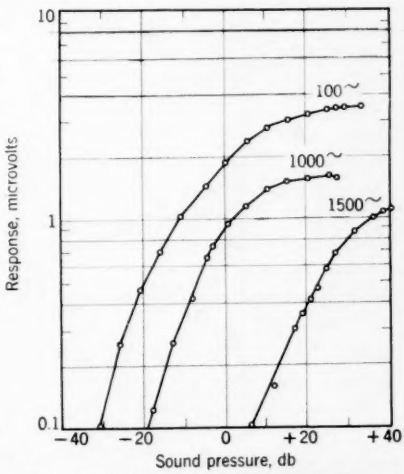

FIG. 2.—Intensity functions. The sound pressure is represented in decibels relative to 1 dyne per square centimeter.

It will be noted that in all these ears the sensitivity is fairly uniform for the low tones, up to 500 or 700 cycles, and then decreases at a rapid rate as the frequency is raised. The general form of the sensitivity function is the same for all three species. In absolute sensitivity the *Chrysemys* and *Pseudemys* groups are similar, and the *Clemmys* group shows somewhat greater sensitivity for the low tones. In general, the measurements made with the dorsally placed electrode show greater sensitivity than those made with the electrode on the cranial wall of the otic capsule, evidently representing better contact with the electrical fields of the sensory cells.

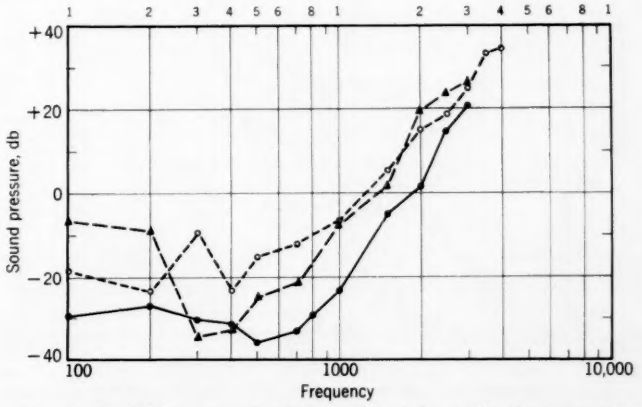

FIG. 3.—Sensitivity curves for three turtles of the species *Chrysemys picta picta*. The sound pressure is represented relative to 1 dyne per square centimeter.

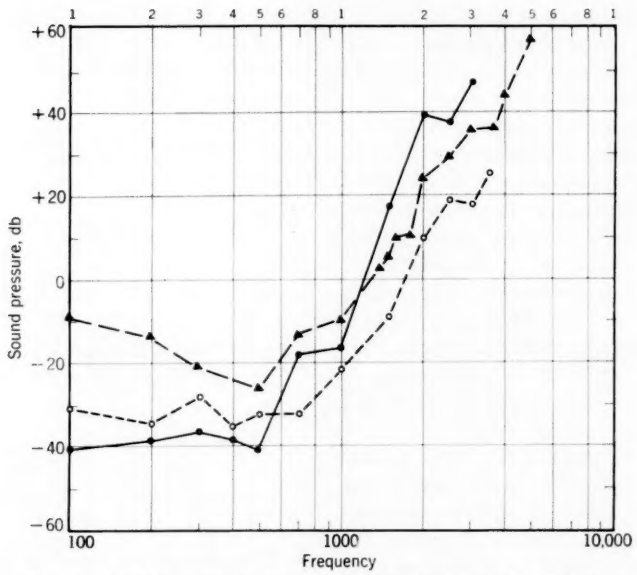

FIG. 4.—Sensitivity curves for three turtles of the species *Pseudemys scripta*.

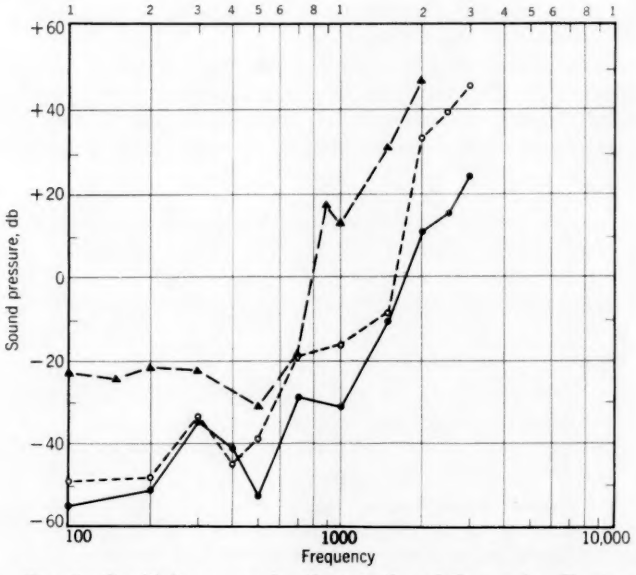

FIG. 5.—Sensitivity curves for three turtles of the species *Clemmys insculpta*.

When the electrode was a platinum foil on the round window membrane, the responses were increased about 5 db over what they were with the dorsally placed needle electrode, irrespective of the stimulus frequency. This fact suggests that the active cells are located in the inferior division of the labyrinth, in the endings of the cochlea, lagena, and saccule.

It is of interest to compare the sensitivity of the turtle's ear as shown in its electrical potentials with that found by the same method in the cat.[9] This comparison is made more fairly if for both forms we use results obtained with the electrode on the round window membrane. On this basis, the turtle's ear measures favorably in relation to the cat's in the low-tone region. For *Chrysemys picta* and *P. scripta* the sensitivity for tones up to 500 cycles is nearly the same as for the cat and for *Clemmys insculpta* it is a little better. In fact, in terms of sound pressure this last species shows about the same sensitivity for these low tones as the cat does in the higher part of the scale—in the region of 1,000–2,000 cycles—where the cat attains its maximum sensitivity.

This near-equality in the responsiveness of turtle and cat to the low tones holds only for the smaller values of sound intensity. As the intensity is raised, the cat's ear increases its output over a wide range, remaining linear from 0.1 microvolt to as much as 200 microvolts for the low tones, whereas for the same tones the turtle's ear rarely produces over 1 microvolt before showing a significant departure from linearity.

Maximum Potentials.—The maximum values of potential obtained for intense stimuli have already been indicated in a preliminary way in Figure 2. More complete data for two ears are given in Figure 6. Some irregularities appear at the two lowest frequencies, but in general the values of the maximums show a progressive decline with frequency. The values are about 8–10 microvolts for the lowest tones and then fall to about 1 microvolt for the highest tones.

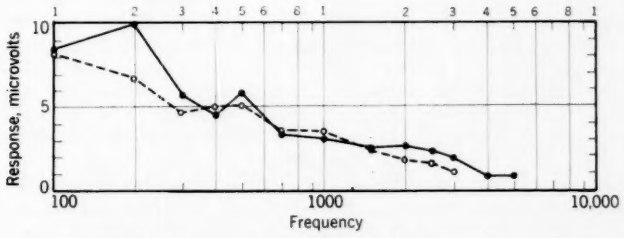

FIG. 6.—Maximum-response curves for two turtles of the species *Chrysemys picta picta*.

These maximum values are to be compared with the ones obtained in mammals, especially in guinea pigs and cats, in which potentials approaching 1,000 microvolts may be measured from the round window membrane for tones below 700–1,000 cycles. The difference of about 100-fold is only in a minor way to be attributed to differences of recording and mainly represents the large increase in the number of sensory cells as we go from reptile to mammal. Beyond 700–1,000 cycles the maximums for the guinea pig decline progressively with frequency, and the same is true for the cat for tones beyond 1,500 cycles. It is interesting to find this same feature

in the performance of the turtle's ear, though with the function displaced downward in the frequency scale.

SUMMARY AND CONCLUSIONS

Electrical potentials in response to sounds were recorded from the inner ear of three species of turtles of the family Emydidae. The recording electrode was given three locations: on the cranial wall of the otic capsule, in the dorsal part of the perilymph space close to the posterior semicircular canal and utricle, and on the round window membrane. These locations are increasingly efficacious in the order named, but the second is the most practical for systematic measurements.

The turtle's ear is highly sensitive, and rather uniformly so, for faint tones in the region up to 500 cycles. For higher tones, however, the sensitivity falls off with great rapidity, and beyond 3,000 cycles a measurable potential can be obtained only at the risk of injury by overstimulation. The sensitivity for low tones is of the same order as that determined by similar methods in the cat, and in one of the species studied (the wood turtle, *Clemmys insculpta*) is nearly equal to that of the cat for higher tones, where this animal has its maximum sensitivity.

For intense stimuli the turtle's ear makes a poorer showing. It has only a limited dynamic range; its potential is a linear function of sound pressure only at low levels, and as the intensity is raised overloading quickly set in. Measured from 0.1 microvolt, the lowest level at which reliable observations have been made, the turtle's range of linearity is about 20 db and its maximum range of response is about 40 db, whereas the cat's range of linearity for low tones is about 66 db and its maximum range is 80 db. It seems reasonable to suppose that the turtle has relatively poor loudness discrimination for any but the faintest sounds.

The general correspondence of the inner-ear potentials of the turtle to the cochlear potentials of birds and mammals evidently represents a basic similarity in the nature and operation of the auditory sense cells.

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CONTENTS

Page

ASTRONOMY.—ELEMENT-BUILDING REACTIONS IN STARS. . . . . *By William A. Fowler and Jesse L. Greenstein* 173

BIOCHEMISTRY.—A COMPARISON OF THE HYDROGENASE ACTIVITIES OF DIFFERENT MICROORGANISMS . . . . . *By Alvin I. Krasna and D. Rittenberg* 180

GENETICS.—GROWTH RESPONSE OF SINGLE-GENE DWARF MUTANTS IN MAIZE TO GIBBERELLIC ACID . . . . . *By Bernard O. Phinney* 185

GENETICS.—THE *q* LOCUS OF *Neurospora Crassa* . . . . . *By Patricia St. Lawrence* 189

GENETICS.—A CASE OF POLYPLOIDY IN DIPTERA . . . . . *By Harrison D. Stalker* 194

MATHEMATICS.—ON COVERING A CIRCLE BY RANDOMLY PLACED ARCS . . . . . *By Aryeh Dvoretzky* 199

MATHEMATICS.—COMBINATORIAL PROBLEMS IN THE THEORY OF GRAPHS. II . . . . . *By G. W. Ford, R. Z. Norman, and G. E. Uhlenbeck* 203

MATHEMATICS.—ON THE LITTLEWOOD-PALEY FUNCTION $g^*(\theta)$ . . . . . *By A. Zygmund* 208

PSYCHOLOGY.—THE SENSITIVITY OF THE TURTLE'S EAR AS SHOWN BY ITS ELECTRICAL POTENTIALS . . . . . *By Ernest Glen Wever and Jack A. Vernon* 213